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CHLOROPHYLL CONCENTRATION AND OXYGEN PRODUCTION IN A SEWAGE OXIDATION POND. PART III. IMPROVEMENT OF OXIDATION POND EFFLUENTS BY ORGANISMS

Charles E. Hazel, et al

Humboldt State College
Arcata, California

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CHLOROPHYLL CONCENTRATION AND OXYGEN PRODUCTION IN A SEWAGE OXIDATION POND

by

Charles E. Hazel
and
John W. DeWitt

PART III

Of Final Report on Project

Improvement of Oxidation Pond Effluents by Organisms

Public Health Service

Research Grant WP-00020-04

John W. DeWitt, PhD
Principal Investigator

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INTRODUCTION

Sewage oxidation ponds (sewage stabilization lagoons) are usually artificially constructed ponds that receive either domestic sewage or a combination of sewage and industrial wastes. An oxidation pond may receive either raw untreated sewage or sewage that has been partially treated by being passed through a "clarifier" or a "trickling filter." Thus, an oxidation pond or a series of them may be used as the only treatment device or as a part of a combination of several devices making up a complete sewage treatment facility.

Oxidation ponds are usually located in suburban areas or near small communities, away from intensive commercial and residential development, where relatively inexpensive land is available. Oxidation ponds vary in size from a fraction of an acre to many acres depending on the amount of organic matter a pond is designed to receive. One of the oldest oxidation ponds known in the United States, and one that is still in operation, is 680-acre Mitchell Lake at San Antonio, Texas, (Smallhorst, 1960). This natural lake has received the sewage from San Antonio since 1901. One of the earliest oxidation ponds in California, and one also still in operation, was built at Santa Rosa in 1924 (Gillespie, 1944). Oxidation ponds have been used throughout the dry and sunny areas of the West for many years, but their value for the practical and effective treatment of sewage has been recognized by sanitarions only since about 1948 (Towne and Horning, 1960). In recent years there has been a rapid rise in the number of ponds constructed and being used for domestic sewage treatment in this country (Towne and Horning, Ibid). Extensive worldwide interest in oxidation ponds is indicated by the questions asked of authorities of the Robert A. Taft Sanitary Engineering Center of the U.S. Public Health Service by foreign visitors to this center (Towne and Horning, Ibid). A comprehensive history of oxidation ponds in the United States, and in some other countries, is presented in the Proceedings of the Symposium on Waste Stabilization Lagoons (U.S. Public Health Service, 1960).

The characteristics of sewage entering a pond depend partly on the type of treatment that the sewage has previously received. Prior treatment may consist of physical settling (clarifying) and/or some degree of biological stabilization. If a pond is used for "secondary" (biological) treatment only, it may receive the effluent from a settling tank (clarifier). In this case, incoming sewage flows into the clarifier where the solids settle to the bottom, and the grease and other floatable substance rise to the surface. The settleable solid material (sludge) is pumped from the bottom of the clarifier into a digester where it is decomposed anaerobically. The fats and other floatable substances, which are removed from the surface of the clarifier, are either dumped into a pond or disposed of by incineration. The supernatant flowing into a pond from the clarifier is still a raw sewage but with a reduced organic waste content. Various biological processes in a pond, constituting secondary treatment, "stabilize" these organic wastes. Generally,

stabilization consists of the breakdown of organic waste materials into inorganic substances, and also the incorporation of such organic waste materials into the cells of organisms. In waste treatment systems which include a pond and a "trickling filter" (rock or other biological filter), the filter usually is incorporated into the system between a clarifier and an oxidation pond. The organic component of the supernatant, trickling over the rocks in a filter, is biologically stabilized to some degree by a characteristic flora and fauna on the rocks. In this case, the filter performs the function of secondary treatment, however, the effluent from such a trickling filter may flow into an oxidation pond where it undergoes further biological treatment. The reduction in the 5-day B.O.D. (biochemical oxygen demand) of sewage passing through a treatment system ordinarily is one measure of the degree of treatment that the sewage has received in the system, because the B.O.D. is proportional to the amount of organic material remaining at any time. Clarifier supernatant ordinarily receives about the same degree of treatment (i.e., reduction in B.O.D. by about 85 per cent) whether it passes through a trickling filter or an oxidation pond.

Stabilization of sewage may occur under anaerobic or aerobic conditions; therefore, oxidation ponds may be classified according to the physiological conditions under which they function, as anaerobic, aerobic, or "facultative" ponds. A pond is classified as facultative when different parts of the pond function anaerobically and aerobically at the same time. The use of (totally or largely) anaerobic ponds for waste stabilization usually is considered objectionable, because gas which is produced by anaerobic processes in such ponds is odiferous. Most oxidation ponds can be classified as "facultative" in that conditions generally are aerobic within the water mass and anaerobic in the bottom sludge.

Generally, the biological stabilization of organic wastes in an oxidation pond involves a wide "spectrum" of plants and animals. Upon entering the pond, the heavy particles still remaining in the sewage after clarification, sink to the bottom. The suspended particles are distributed throughout the pond by water currents, and the decomposition of this suspended matter is carried out primarily by aerobic bacteria. Many decomposer bacteria are able to utilize only specific organic compounds; therefore, there are successions of different bacterial species involved depending on the stage of breakdown of the sewage. Much of particulate matter and many bacteria are filtered from the water and used as food by filter-feeding animals such as cladocerans, ostracods, rotifers, and protozoans. By feeding upon the particulate sewage matter, these organisms aid in the stabilization of sewage.

Since large quantities of oxygen are necessary for the proper functioning of an aerobic oxidation pond (Oswald and Gotaas, 1954), algae are organisms of primary importance in the ecology of such a pond. The amount of atmospheric oxygen that can pass by diffusion into a

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pond is limited to a maximum of about 40 pounds per surface acre per day; this amount is reportedly insufficient to maintain aerobic conditions in oxidation ponds (Oswald, 1960). Photosynthesis, therefore, must supply most of the oxygen needed. The algae that produce this oxygen exist in a symbiotic relationship to the aerobic decomposer bacteria; that is, the algae are dependent upon bacterial decomposition of organic matter for the carbon dioxide they use in photosynthesis and for the other nutrients necessary for their growth and reproduction. Using photosynthetic oxygen as a hydrogen acceptor, aerobic bacteria oxidize the organic wastes, thus releasing carbon dioxide and other nutrients. Using light as an energy source, the algae utilize these nutrients to form new protoplasm. In this process there is a release of oxygen which is then used by the bacteria, completing a basic carbon and nutrient cycle. The total bacterial demand for oxygen for aerobic decomposition is dependent on the quantity and quality of the organic wastes added to the pond. Two sources of energy are available to the pond community, sunlight, and the organic matter flowing into the pond. The degree of utilization of these energy sources under aerobic conditions is dependent on the symbiotic relationship between the algae and bacteria.

Much of the putrescible organic matter that enters a pond leaves it in a stabilized form through "outflow." Outflow may include the dissolved and suspended substances lost from the pond via its effluent, loss of gasses through diffusion to the atmosphere, and the removal of organic matter from the pond by birds, or the emergence of aquatic insects. Some of the organic matter which settles to the bottom of a pond is eventually removed from the pond, after being resuspended, through outflow mechanisms. In a general sense, most of the sewage flowing into an oxidation pond leaves the pond in the form of plant, animal and bacterial cells, water, the gaseous products of cellular metabolism in the pond, and dissolved inorganic and organic nutrients (Hawks, 1960, and Simpson, 1960).

One of the main problems in the use of aerobic oxidation ponds for the treatment of sewage is the maintenance of aerobic conditions in the open water of these ponds. Since most of the oxygen required for the maintenance of suitable conditions is derived from photosynthesis by photoplankton, any factor, biological or physical, which directly or indirectly affects the oxygen production of the phytoplankton may also affect oxygen conditions in the ponds. Examples of such factors are light intensity, the abundance of organisms which graze on phytoplankton, the supply of nutrients, and the supply of oxygen for aerobic decomposition. Since there must be larger number of factors which may alter the size or the physiology of phytoplankton populations, one of the major problems in studying oxidation pond ecology is deciding which factors are of major importance and which factors may have some indicator or predictive value.

The relationships of algae to the sewage treatment process in oxi-

dation ponds have been studied intensively on a pilot plant scale at Richmond, California, (Ludwig, Oswald, and Gotaas, 1952; Gotaas, Oswald, and Golucke, 1954). Silva and Papenfuse (1953) and Allen (1955) studied algae in oxidation ponds and oxidation pond ecology in central California. Oswald (1960) discusses oxidation pond functions and design as determined from pilot plant studies at Richmond, California. Most other reported field studies performed on sewage oxidation ponds have dealt more specifically with the degree of treatment received by the waste waters, as measured by B.O.D. reduction and reduction in the "most probable number" (M.P.N.) of coliform bacteria, rather than with the details of pond ecology. Studies of the former kinds are reported by Wilson, McDermott, and Livingston (1960), Neal, and Hopkins (1956), and the California Department of Water Resources (1958). Chemical and physical analyses of oxidation pond effluents have been included in various kinds of investigations.

The ability of herbivorous grazers, such as Daphnia, to decimate a phytoplankton population has been reported by Oswald, Golueke, and Gotaas (1959). Bartsch and Allum (1957), in a study of oxidation ponds in North Dakota, made measurements of insolation, oxygen production and chlorophyll. This study (Bartsch and Allum, ibid.) is the only one known in which measurements of oxygen production and the distribution of chlorophyll have been made under field conditions. In the ponds studied, these workers found shallow euphetic zones, less than one meter in depth, and chlorophyll concentrations that varied with depth. It has been reported (Kosminski, 1938; Manning and Juday, 1940; Currie, 1958) in studies of extremely divergent aquatic habitats that the quantity and the vertical distribution of chlorophyll are related to the depth of the water and the intensity of the light available for photosynthesis.

The main objectives of the study being reported herein were to gain fundamental knowledge of the dynamics of the populations of phytoplankton and of some of the principal environmental influences on the dynamics of these populations, and to determine primary production in a sewage oxidation pond. Preliminary measurements were made of short-term changes in phytoplankton concentrations at various depths in the pond and at different times of day. These short-term changes were then considered in designing a comprehensive phytoplankton sampling program to meet the overall objectives of the project. Along with samples collected to estimate the size of the populations of phytoplankton, simultaneous measurements were frequently made of solar radiation, the standing crop of Daphnia magna, ash-free dry weight of suspended matter, gross oxygen production, and community respiration.

The study period was from February 1960, through December 1961. During the entire period, emphasis was placed on the collection of samples of algae and on spectrophotometric determinations of Chlorophyll a, b, and c in the samples. During the spring of 1960, samples of water were taken near the surface of the pond, and aliquots were used for pigment analysis and for the determination of the ash-free dry weight of

the suspended solids. In the late spring and during the summer and fall of 1961, water samples for pigment analysis were taken in conjunction with samples of Daphnia magna.

From July through November, 1960, phytoplankton samples were collected at four depths at various stations to determine the vertical distribution of the phytoplankton. From these samples, it appeared that the vertical distribution of phytoplankton followed certain patterns. During June and July 1961, samples were collected at different times and depths during the day to ascertain the occurrence of the suspected patterns. Surface light intensities, gross oxygen production, and community respiration were also estimated during June and July, 1961. The gross production of oxygen per milligram (mg). Chlorophyll a per hour and community respiration (oxygen consumption) values in mg oxygen per mg Chlorophyll a per hour were computed from Light and Dark bottle analyses. The concentrations of Non-astacin (carotenoid) pigments in some of the samples were determined. Non-astacin pigment concentrations were used to obtain Chlorophyll a: Non-astacin pigment ratios which may indicate some aspects of the physiological condition of phytoplankton populations.

STUDY AREA

Geographic Location

The sewage oxidation pond that was studied is part of the sewage treatment facility of the City of Arcata, Humboldt County, California. Arcata is in north-coastal California at the north-east end of Humboldt Bay on U. S. Highway 101, about seven miles north of Eureka. The pond is located on the south edge of the city on the tidal flats of Humboldt Bay.

Climate

The Humboldt Bay region has a maritime climate characterized by high humidity and moderate temperatures. May through September is the dry season; however, the 50-year mean annual rainfall at Eureka is 39.49 inches. During the summer, a typical day is characterized by fog or low clouds in the morning, with clearing before noon, and fog or clouds again in the evening. During the year, about 75 per cent of the days are cloudy or partly cloudy. The 50-year mean pattern is 82 clear, 103 partly cloudy, and 180 cloudy days per year. The usual annual range in temperature is from 2 to 24° c. with a 4°c. to 5° c. variation in any given day. The prevailing winds are from the north-east with a mean hourly speed of 7 m.p.h., but often exceeding 40 m.p.h. The climatological data given are for the Eureka Weather Station (U. S. Department of Commerce, 1960), located on Humboldt Bay about 5 miles south of Arcata.

Associated Sewage Treatment Facilities

The Arcata sewage treatment plant and the oxidation pond, designed to handle the domestic wastes of up to 15,000 persons, were constructed in 1957. In 1960, at the beginning of the present study, the population actually being served by these facilities was about 7,500. Ordinary domestic sewage and some industrial wastes, particularly those of plywood and pressed chip-board mills, enter the sewage collection system. In addition to the normal flow of sewage and wastes a large amount of run-off (ground) water infiltrates the collection system during the rainy season.

Before entering the oxidation pond, all wastes ordinarily undergo primary treatment which consists of aeration and clarification. In the latter process, the settleable solids are pumped from the clarifier into a two-stage digester, and the floating material is skimmed into a grease trap. The grease trap is emptied periodically into the oxidation pond. The supernatant from the clarifier flows by force of gravity into the oxidation pond. When the total sewage flow into the plant exceeds 3,000 gallons per minute (g.p.m.), the primary treatment facility is by-passed, and the sewage flows directly into the pond. Excessive infiltration of ground water into the sewage collection system during periods of high rainfall is responsible for the increased flows which necessitate by-passing.

Description of the Oxidation Pond

The pond was constructed in 1957 on a tidal mudflat by building up an enclosing dike of mud dredged from the bay. With the pond surface at an elevation of 6.5 ft. above the mean low water level, the surface area of the pond is about 59 acres. At this elevation the mean actual depth of the pond over most of the area excluding a borrow trench 6 to 8 ft. deep along the inside of the dike is about 3 ft. When the surface area is 59 acres, the pond contains an estimated 260 acre-feet of water. The approximate normal detention time, or flow through time, is 21 days when the inflow of sewage is 3.8 million gallons per day (m.g.d.), and it is 210 days when the flow is 0.4 m.g.d. (City of Arcata Department of Public Works, 1959). There is a sparse growth of vegetation on the dike, and there is some emergent vegetation around the edge of the pond. Figure 1 shows the general shape of the pond and the points of inflow and outfall. The inflow enters the pond at its bottom approximately 25 yards from the shore. A flap gate at the outfall prevents the intrusion of bay water into the pond at high tide.

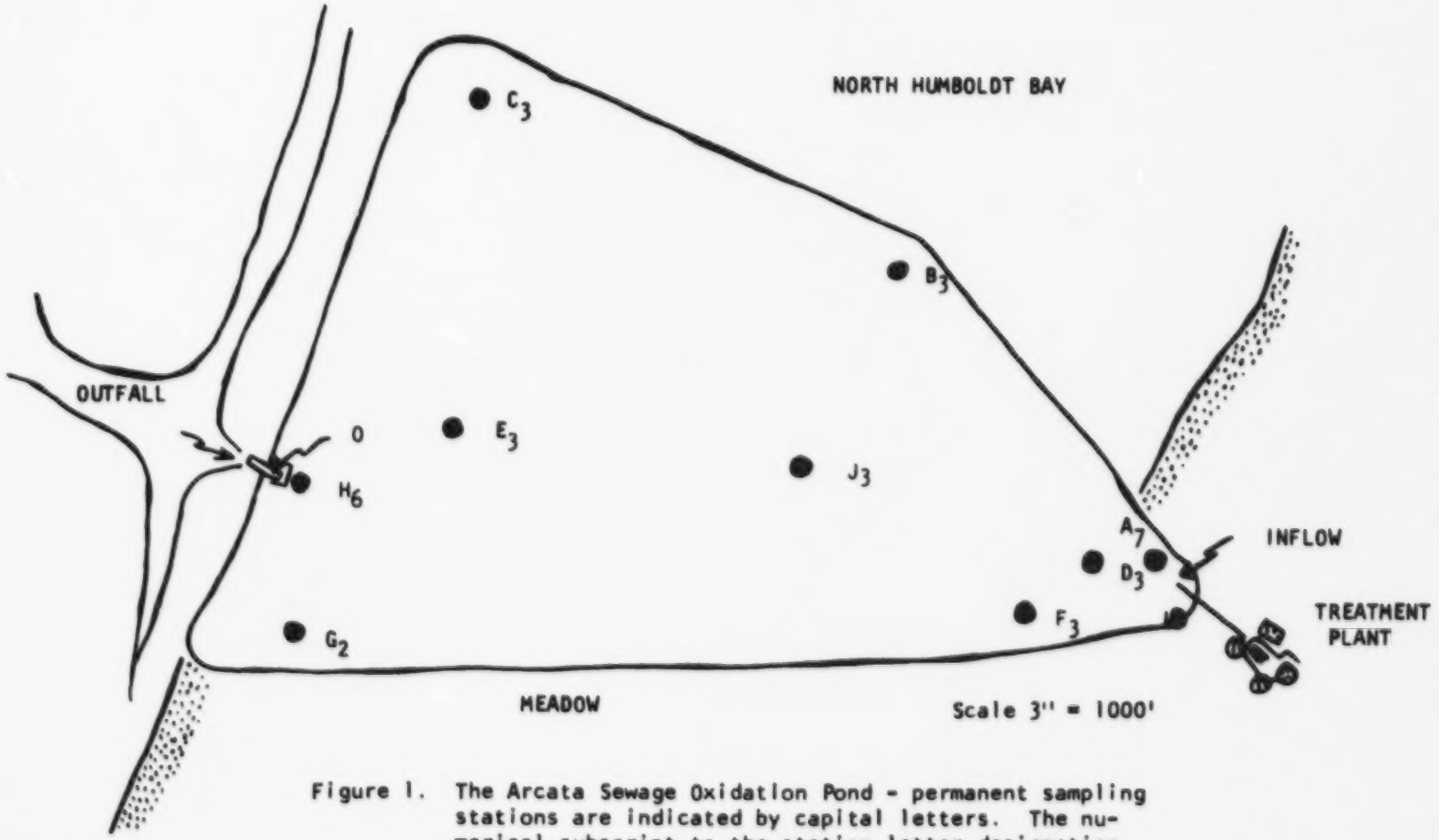

Figure 1. The Arcata Sewage Oxidation Pond - permanent sampling stations are indicated by capital letters. The numerical subscript to the station letter designation indicates the approximate water depth in feet at a station.

METHODS AND MATERIALS

Sampling

During the early (February through June, 1960) part of the study period, surface water samples for pigment analysis were obtained by pushing a collection bottle (usually a 300 ml B.O.D. bottle) about six inches beneath the pond's surface. Samples from near the bottom of the pond were obtained in the same period in a similar manner except that the collection bottle was fastened to a pole. After pushing the collection bottle to the bottom of the pond, the stopper, and the bottle was allowed to fill. Later water samples were taken with a sampler made from an aspirator bottle (Figure 2). The technique used in obtaining samples with this sampler is as follows: A screen is placed over the inlet spout of the bottle to keep out large organisms and particles of detritus. The air outlet tube is pinched closed, and the water sampler is submerged to the desired depth and slowly moved horizontally for a distance of about one foot. A clamp is released from the air outlet tube allowing the bottle to fill. After the bottle has filled, the air-outlet tube is again clamped closed, and the sample is brought to the surface. The screen is replaced by a delivery tube for drawing out samples of water. When drawing a sample, enough water is allowed to flow from the bottle back into the pond to clear the delivery tube of air and foreign matter. After being cleared, the delivery tube is placed at the bottom of a 300 ml B.O.D. bottle and the bottle is filled without undue agitation.

Phytoplankton samples were collected at depths of 10, 25, 40, and 70 cm. at fixed stations (Figure 1) in the pond. Stations D, J, E, H, and G, in this order, were in a generally direct path from the region of the inflow to the outfall. Stations A, B, C, F, and G were located around the edge of the pond about 50 feet from the shore; and station I was located at the boat dock 2 feet from the shore. Each station was permanently marked with a 2 inch steel pipe driven into the mud and extending above the water's surface. Samples were collected from a boat fastened to these station markers, except at stations I and O. Sampling at station I was done from the boat dock, and the samples at station O were collected from the water flowing over the dam boards.

Pigment Analysis

For pigment analysis, 300 ml B.O.D. bottles were filled from the aspirator sampler to about two-thirds of their capacity and placed in the dark in a closed plywood box. Euglena sp. (the predominant alga encountered during the study) had a tendency to clump in the palmella stage inside the bottles. Because of this clumping, it was necessary to shake the bottles frequently between the time of collection and the time of analysis in the laboratory to insure a homogenous distribution of organisms within the bottle. If the suspended particulate matter in the samples was not removed shortly after the samples arrived at

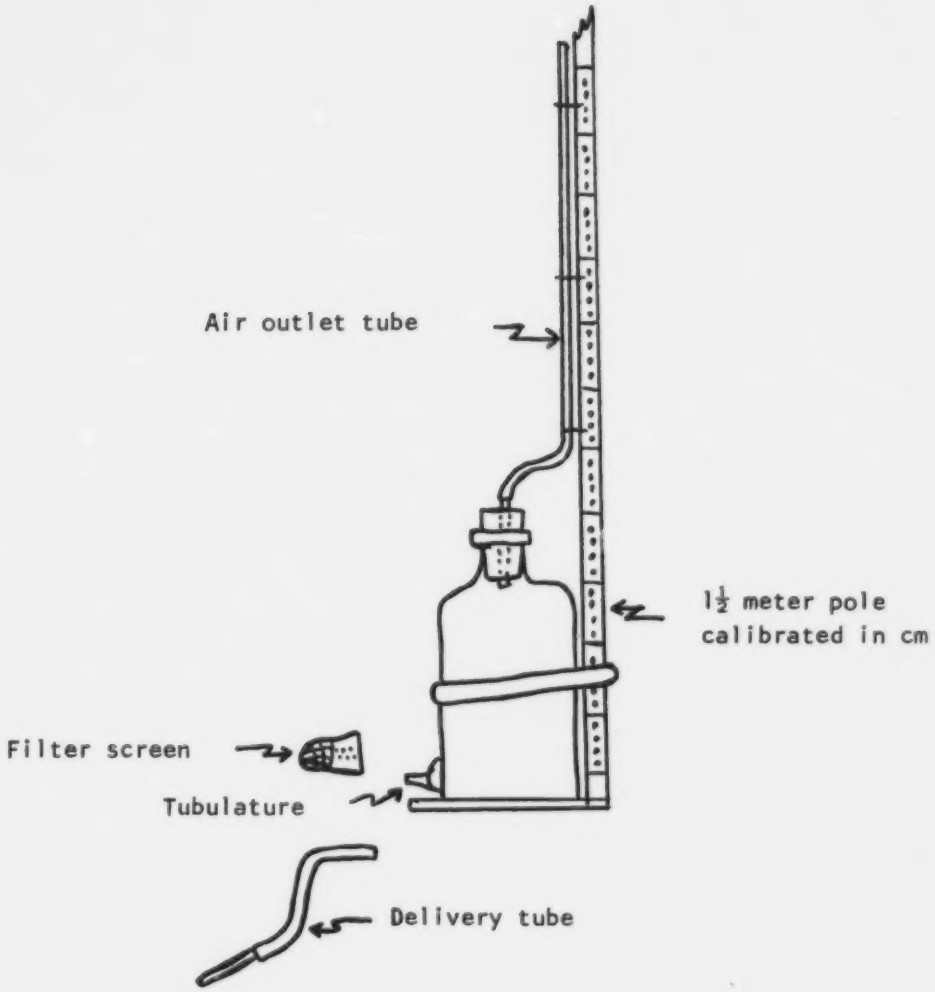

Figure 2. Schematic representation of the aspirator-bottle water sampler used for sampling phytoplankton.

the laboratory, they were stored in the dark in a refrigerator at 3°C. for periods up to 24 hours.

Two methods of separating suspended solids from water samples were used, and a different drying temperature for the residue obtained was used for each method. Before January 1961, the suspended matter was separated from samples for pigment analysis using a Foerst continuous centrifuge, and this residue so obtained was dried at 52° C. After January, 1961, the "millipore" filter method (Creitz and Richards, 1955) was used for the separation, and drying of the residue on the filters was done at 3° C. Samples were filtered within one hour after collection. Quantitative estimations of pigment concentrations were made using the spectrophotometric technique described by Richards with Thompson (1952).

In separations completed with the centrifuge, about 0.1 gram of powdered magnesium carbonate was added as a buffer to the samples of water to prevent the sample from becoming acid and inducing a molecular change in Chlorophyll a, and thus the formation of pheophytins. Samples were centrifuged three times at a flow rate through the centrifuge of one liter per 7 minutes. After centrifuging, the inside of the centrifuge collection cup was scraped with a rubber policeman, and the residue was washed from the cup into a 50 ml clinical centrifuge tube. This suspension was centrifuged for 10 minutes and then decanted. The residue obtained was dried in the dark for 12-18 hours at 52° C. The dry residue was then broken up with a dissecting needle and 90 per cent acatone was added to the residue to extract the pigments. The acetone was prepared by diluting reagent grade acatone of 99.5 per cent concentration with distilled water. Pigments in the residue were extracted by steeping in the dark for 18-24 hours at about 3° C. After steeping, the acetone-pigment solution was well shaken and then centrifuged for about 10 minutes. The acetone solution was then decanted into one centimeter spectrophotometer cells, and absorbencies were measured at the required wave lengths with a Beckman Model D.U. spectrophotometer for the quantitative determination of chlorophylls a, b, and c, Astacin, and Non-astacin pigments.

Two sources of error in the pigment analysis technique, using centrifugation and drying at 52° C., are known to exist. It was assumed that drying at 3° C. as recommended by Strickland (1960) gave the most accurate information, and the results from the samples dried at the two temperatures (3° and 55° C.) were compared statistically. Results of experiments on determining the nature of these errors are summarized in Appendix II. The first and probably most important error was that the suspended solids were not entirely separated from the water sample during centrifuging. Secondly, there were changes in some of the pigments due to heating during the drying process as indicated by significant differences between the measured concentrations of chlorophyll c and the Astacin and the Non-astacin pigments when dried at 55° C. from that was assumed to be the normal concentration. Duplicate sets of samples were dried at the two temperatures used during the study

In the separation of suspended solids with the "millpore" (MP) filter, NA 0.45 micon filtere instead of the type AA filters recommended by Creitz and Richards (1955), were used since the recommended filters were not available. The volume of pond water that could be drawn through a filter in a reasonable length of time varied between 25 and 100 ml. Although no quantitative determinations were made, the volume of water that could be filtered in a given period of time appeared to be independent of the chlorophyll concentration. The largest aliquot that could be rapidly filtered, such that clogging of the filter did not occur, was used. After filtering, the M.P. filter was folded with the residue inside and fastened with a paper clip inside a folded piece of ordinary filter paper. The samples were then dried in a dessicator in the dark for 18-24 hours at a temperature of 3° C. If steeping was not done immediately, the samples were stored in the dark in a dessicator at -10° C. for periods up to one month. Steeping of the filtered residue was done in a ground glass stoppered 50 ml centrifuge tube in the dark for 18-24 hours at 3° C.

Light and Dark Bottle Determinations

The Light and Dark Bottle (L&DB) method, originally described by Gardner and Grann (1927), was used to measure gross production of oxygen and community respiration. This method has previously been used by Bartach and Allum (1957) for measuring the production of oxygen in sewage oxidation ponds. Using the L&DB technique, estimates of *in situ* gross production of oxygen and community respiration were made. In this method, the oxygen concentration of an aliquot of a water sample taken from the pond is determined initially to ascertain the oxygen concentration at the time the sample was collected. The dissolved oxygen content of aliquots incubated in the Light and Dark bottles was determined after an *in situ* incubation period of 2 to 4 hours. The concentration in the Light bottle minus the concentration in the corresponding Dark Bottle (LB-DB) was taken as the gross production of oxygen. The concentration in the initial aliquot minus the concentration in the Dark bottle (LB - DB) was taken as respiration.

Both the Light and the Dark aliquots were incubated in 300 ml. glass stoppered (B.O.D.) bottles. The Dark bottles were covered with a double layer of black canvas. An excess of canvas was left at the top of the bottle, and this canvas was folded over the top after the stopper was in place. A 3/8 inch wide rubber band was stretched over each bottle, Light and Dark, to hold the stoppers tightly in place. The bottles, attached to plastic coated wire clothes line, were suspended in the pond at the desired depths, however, the Light and Dark bottles were suspended on separate lines. This method of suspension is schematically illustrated in Figure 3. To eliminate any shadowing from a station marker, the bottles were suspended on the south side of the marker.

Each Light and Dark bottle was filled from the water sampler by placing the delivery tube at the bottom of a bottle and allowing the

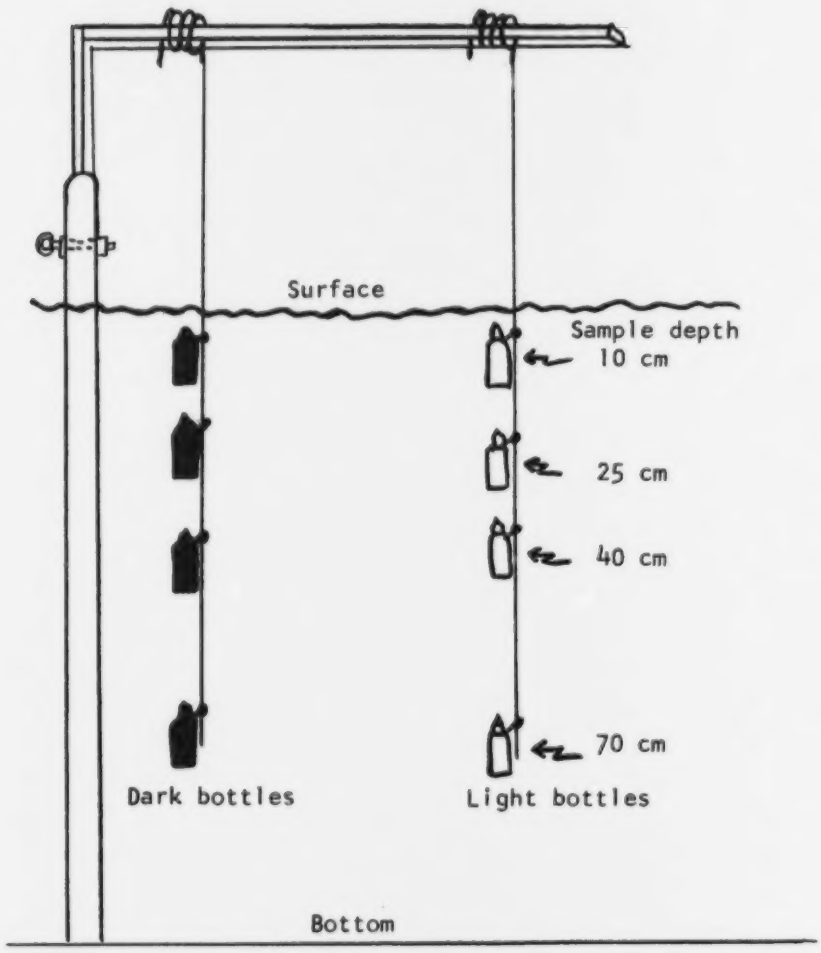

Figure 3. The method used to suspend light and dark bottles in the pond.

bottle to over-fill. After being filled, each bottle was stoppered with a ground glass stopper coated with stopcock grease. After a complete set of samples had been collected, the individual bottles containing the aliquots were fastened to the line at their respective sampled depths. After this, the dissolved oxygen in the initial aliquot was fixed for oxygen analysis. Upon being retrieved from the pond, and after the incubation period, the dissolved oxygen in the Light and Dark bottles was fixed. The midpoint in time between the beginning and the end of a sampling or retrieval sequence was the reported sampling time. These time intervals varied from 5 to 15 minutes. All the samples that were fixed for dissolved oxygen determinations were returned to the laboratory for titrations. When the titration was not done within an hour after returning to the laboratory, the fixed samples were stored up to 36 hours in the dark at 3°C. before being analyzed.

Determination of Dissolved Oxygen

Two different modifications of the Winkler method for the chemical fixation of dissolved oxygen were used during the study. After November 1960, the Pomeroy Kirschman-Alsterberg (P.K.A.) modification was put into use (American Public Health Association, et al., 1955). Before this time the Sodium Azide modification was used. In the P.K.A. method, sodium iodide replaces potassium iodide in the alkaline-iodide reagent. Two ml of each fixing reagent was used in the fixation process. It was found that the Sodium Azide method indicated dissolved oxygen concentrations that were lower than those obtained with the P.K.A. method for the same water samples when the concentrations were above 15 mg/L. As measured using the P.K.A. modification, dissolved oxygen concentrations up to 35 mg/L were observed in Light bottles. Using the method described by Ellis, Westfall, and Ellis (1943), the fixed aliquots were titrated with 0.0125 normal sodium thiosulfate which was standardized with potassium dichromate.

Sources of Error in the Light and Dark Bottle Method

Strickland (1960) indicates that the Light and Dark Bottle (L&DB) method is not applicable to waters of high organic content, because the Winkler method for measuring dissolved oxygen was of insufficient precision in such waters. In the present study the use of the P.K.A. modification of the Winkler method may have partially overcome this difficulty. In any case, the LADS method was the only means available for measuring gross photosynthesis. However, difficulties were encountered in the use of the LADS method in the Arcata Pond. Supersaturations of didissolved oxygen developed in some aliquots being incubated, causing gas bubbles to form within the incubation bottles. Although these initial aliquots were at less than the air-saturation point of dissolved oxygen, supersaturations developed under some circumstances within a two or three hour incubation period. The escape of gas bubbles when a stopper was later removed for the introduction of the fixing reagents would lower the measured quantity of dissolved oxyten. A technique of handling bottles was developed to minimize the escape of gas bubbles while the

fixing reagents were being introduced. The bottles suspected of containing trapped gas bubbles were turned upside down and rapped sharply with a wooden stick three or four times. This action dislodged the bubbles adhering to the sides of the bottle and around the base of the stopper, allowing them to float to the bottom of the inverted bottle. Righting the bottle, but retaining it at a slight angle from the perpendicular, trapped the bubbles in the bottle's shoulder. If held at a proper angle, the stopper could be removed from the bottle and the fixing reagents introduced without bubbles escaping. The stopper was replaced and the bottle was shaken vigorously. After the floc had settled through about 1/3 of the bottle's volume, sulfuric acid was added.

A second difficulty and possible source of error was due to the clumping of the algae. During the period of incubation, Euglena sp. in the palmella stage would clump forming large gelatinous masses which adhered to the sides of the bottles. Not all of the algae within a bottle were involved in the clumps. Clumping occurred most frequently, and the clumps were larger, in bottles incubated near the surface of the pond, especially during periods of high light intensity, but it occurred in all samples with high concentrations of algae. Although the effect of such clumping on the rate of photosynthesis is unknown, it is assumed that it could only result in a diminished average rate per alagal cell.

It is assumed that the environments within the bottles were not the same as the pond environment; therefore, the L&DB is only an estimation of actual in situ oxygen production. The formation pf palmelloid colonies of Euglena may be indicative of a restrictive environment in the bottle. The Euglena in samples from the pond were pre dominantly of the motile form. Because the palmella stage settles toward the bottom of the water mass, these forms probably were not obtained in large numbers from the open water of the pond. Also, a substrate, such as the bottle wall, may aid in the formation of palmelloid colonies. The community of organisms within a bottle was subjected to a closed environment. A principal and recognizable difference between bottle and pond environments is the lack of water circulation in a bottle, a phenomenon characteristic of ponds. Wind generated currents often mix pond water causing circulation of nutrients required for photosynthesis and dissipation of metabolic wastes.

The differences in community respiration, between the Light and Dark bottles, caused by differences in rates of growth, reproduction, and mortality of the algae present may be highly significant (Edmondson and Edmondson, 1947), as may errors introduced by differential bacterial growth occurring in the bottles (Pratt and Berkson, 1958). No attempt was made in the present study to quantitatively estimate the degrees of error resulting from the above or other causes.

The Determinations of the Ash-free Dry Weight of Suspended Particulate Matter.

Duplicates of pond samples used for pigment analysis were run

through a Foerst continuous centrifuge to obtain the suspended particulate matter for ash-free dry weight determinations. The residue was washed from the centrifuge collection cup with distilled water into a Coor's crucible which had been previously weighed. A standard procedure was used for all weighings. After removal from an oven, the crucible was cooled for one hour in a dessicator. The crucible was removed from the dessicator and immediately weighed to the nearest 0.0001 gram on a "Chain-O-Matic" analytic balance. After drying at 105° C. overnight, the crucible and the residue were weighed. After weighing, the residue was ignited at 600° C. for one-half hour and allowed to remain in the oven to cool to aboue 200° C. After being removed from the oven, enough 5 per cent ammonium carbonate solution was added to saturate the residue. The moist residue was returned to the oven and dried at 105° C. After drying for at least 2 hours the crucible was cooled in a dessicator and then weighed to determine the ash weight. The dry weight minus the weight of the ash was recorded as ash-free dry weight (organic weight or weight of volatile solids).

RESULTS OF PIGMENT ANALYSES

Chlorophyll a: Daily Variations in Relation to Depth

The quantity and the distribution of chlorophyll in a column of water varies with the depth of the water and with the intensity of the light that is available for photosynthesis. Kosminksi (1938) found variations in the vertical distribution of chlorophyll in some Wisconsin lakes, and Currie (1958) found variations similar to those reported by Kominski in the Eastern Atlantic Ocean. Bartsch and Allum (1957) at Kodoka, South Dakota, observed daily variations in the concentration of Chlorophyll a at given depths in a raw sewage lagoon. In the Arcata pond, variations in concentration of Chlorophyll a and of Non-astacin pigments (carotenoids) at different depths, time of day, and surface light intensities were investigated during 1960 and 1961. The data from all pigment analyses are included in Appendix I.

The distribution of phytoplankton in a pond is influenced by these factors: reproduction, growth, mortality, motility, water currents, and light intensity. Reproduction and autotrophic growth, which may result in an increase in the concentration of chlorophyll, unless the rate of algal cell mortality is too high, are directly influenced by light intensity and the concentration of nutrients in the euphotic zone. Figures 4 and 5 illustrate a general relationship between changes in the concentrations of Chlorophyll a observed at the sampled depths were plotted against time of day and surface light intensity, there was an observed correspondence between increasing light intensity and an increasing concentration of Chlorophyll a although the rate of increase varied with depth, such that as the surface light intensity became greater the difference in concentration of Chlorophyll a between the two observed depths became larger.

Thirty sets of samples for pigment analysis were collected from the pond at depths of 10, 25, 40 and 70 cm during the study period. All of these samples were collected at station E with the exception of two sets collected from station D. The vertical distributions of Chlorophyll a at stations D and E were similar at approximately the same times (Figures 6 and 7). Different patterns of vertical distribution of Chlorophyll a were observed, and of these patterns two markedly different ones are illustrated in Figures 6 (no change in concentration with depth) and 8 (a marked change with depth). The other patterns observed varied between these two extremes (see Figures 8 through 9). Different vertical distributions of Chlorophyll a appear to occur at different levels of surface light intensity and different levels of abundance of plant pigments insofar as the concentration of pigments affect light penetration into the water.

In cases where Chlorophyll a was evenly distributed from the surface to the bottom of the pond, surface light intensities were approximately

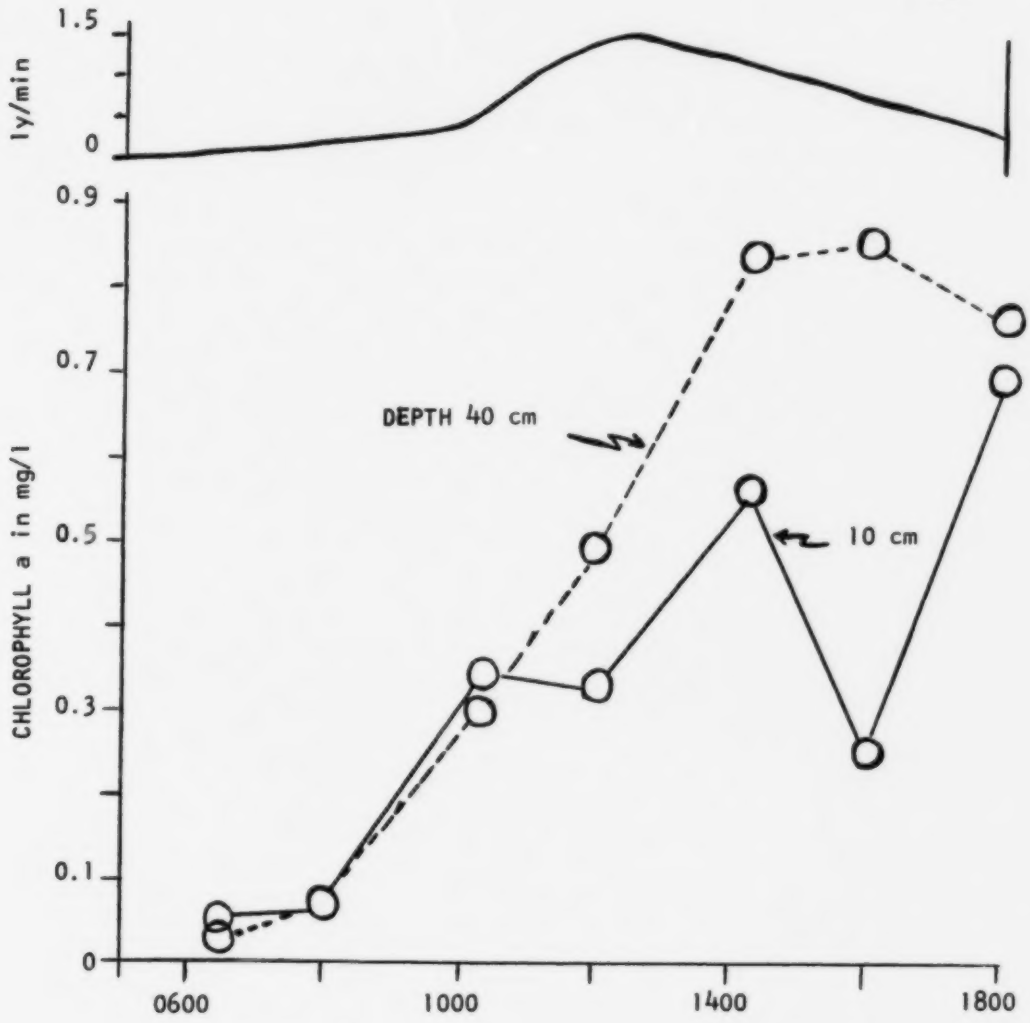

Figure 4. The concentration of Chlorophyll a at two depths, 10 and 40 cm, illustrating the variation in pigment concentration at these depths in relation to surface light intensity at station E on June 21, 1961.

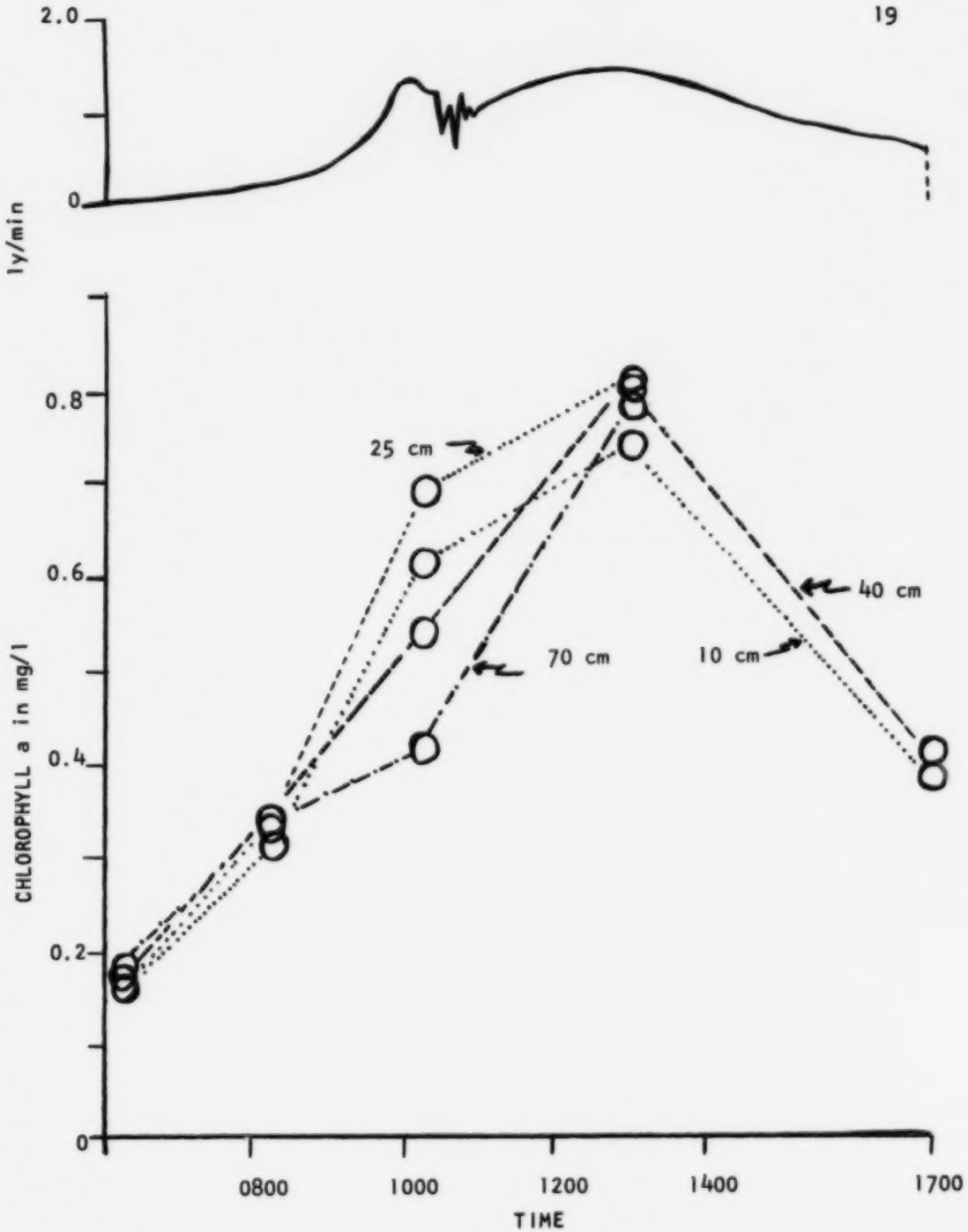

Figure 5. Chlorophyll a concentrations at four depths, 10, 25, 40, and 70 cm, and surface light intensity at station E on July 28, 1961

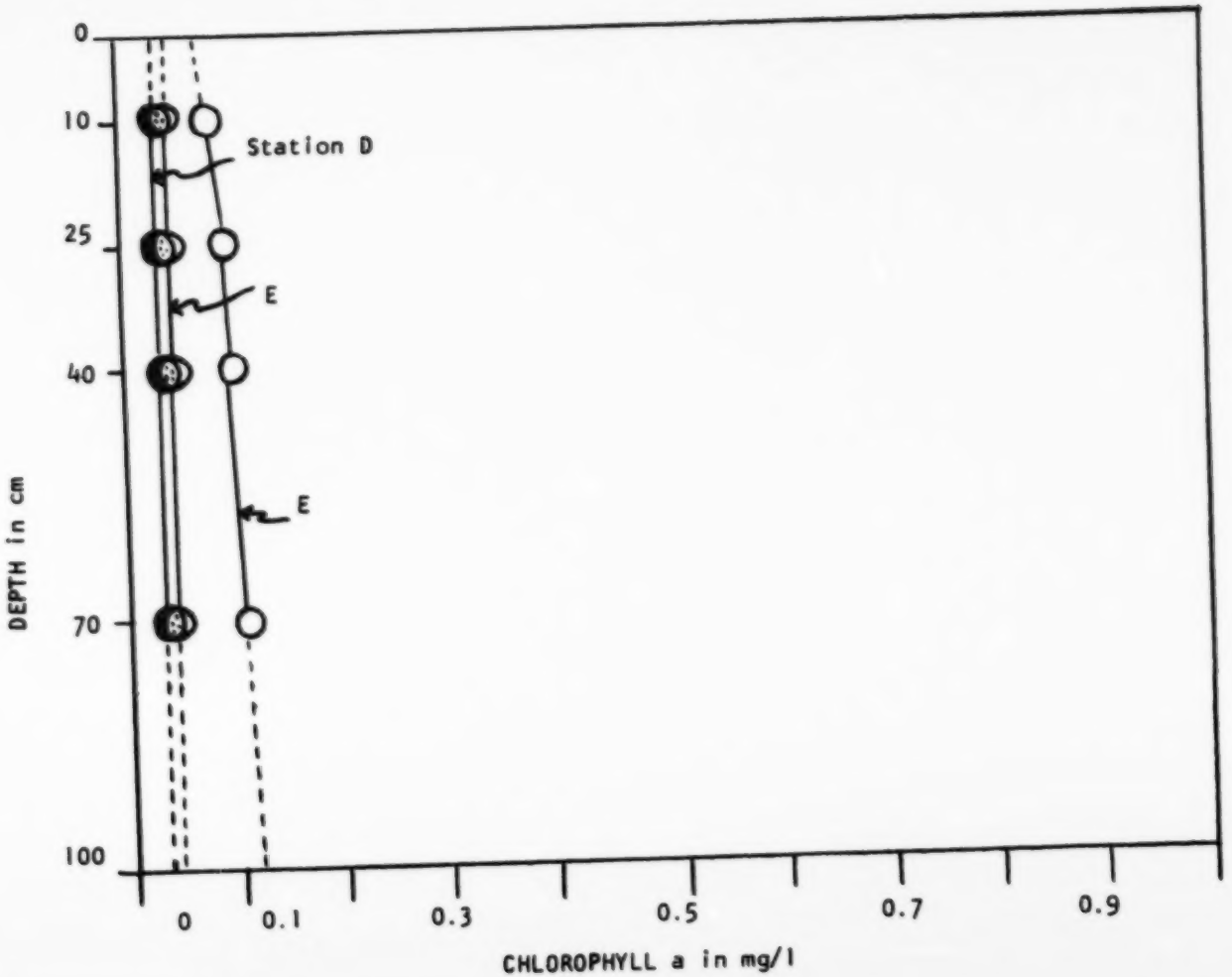

Figure 6. Chlorophyll a concentration in relation to depth at stations E and D in September 1960.

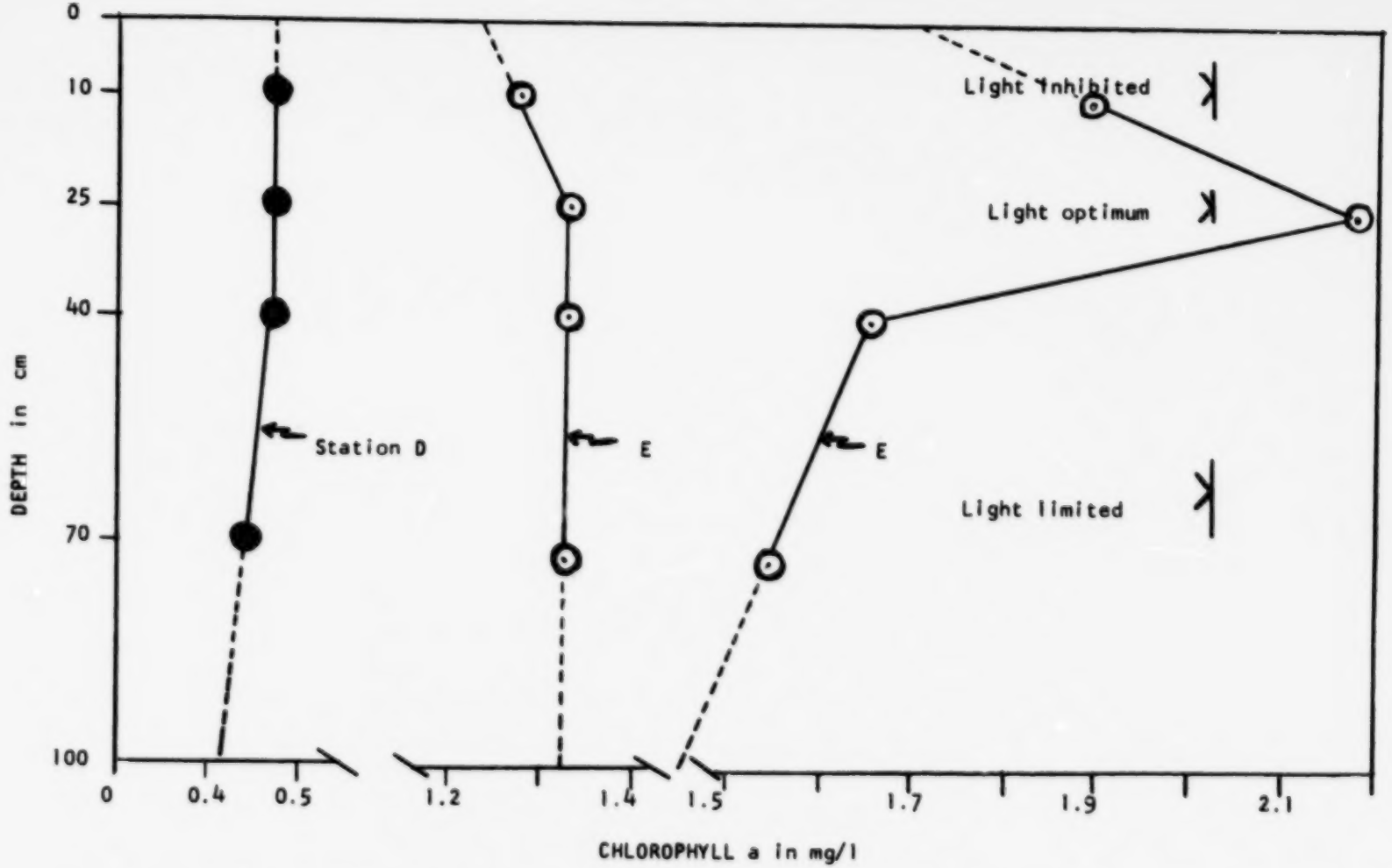

Figure 7. Chlorophyll a concentration in relation to depth at stations D and E in October 1960.

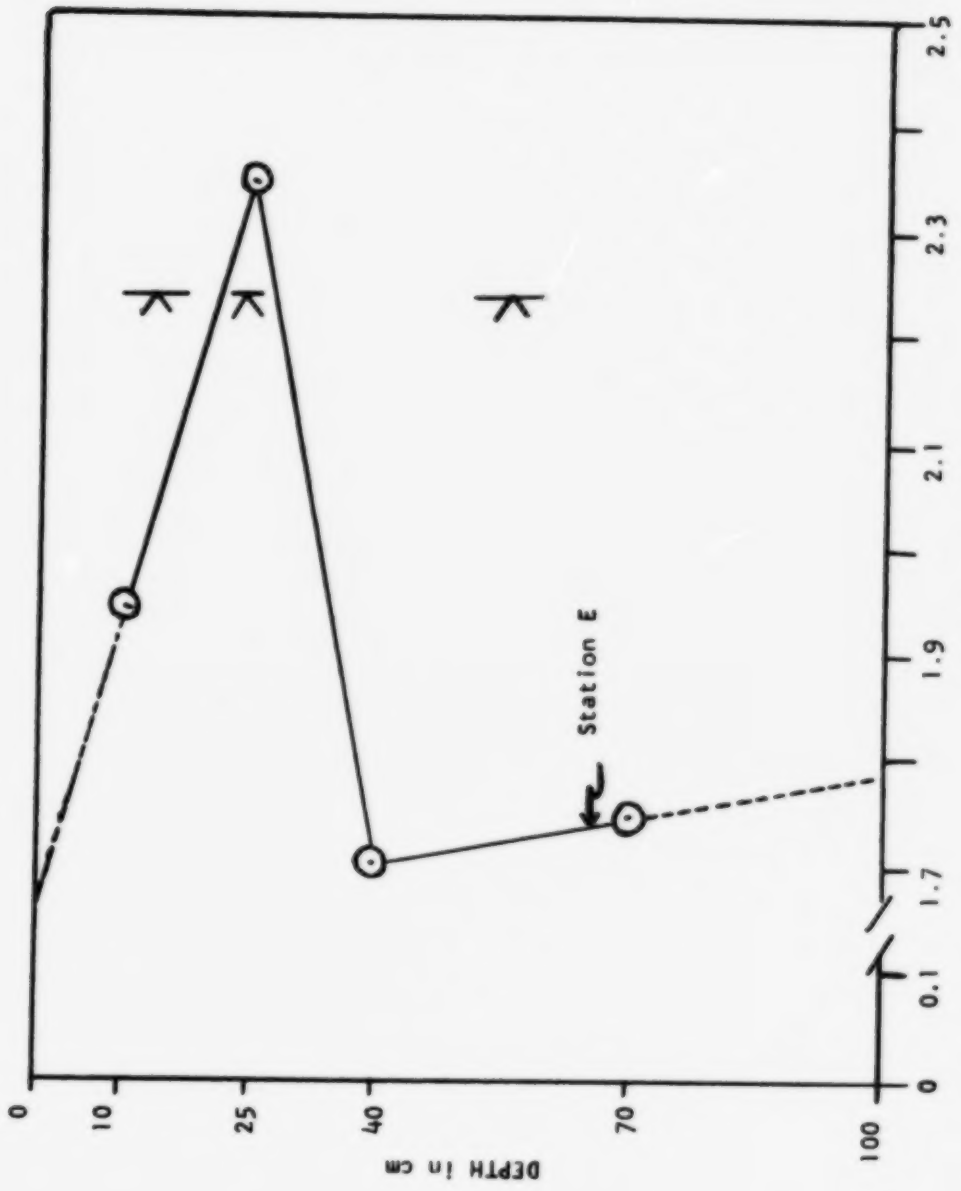

Figure 8. Chlorophyll a concentration in relation to depth at station E in November 1960.

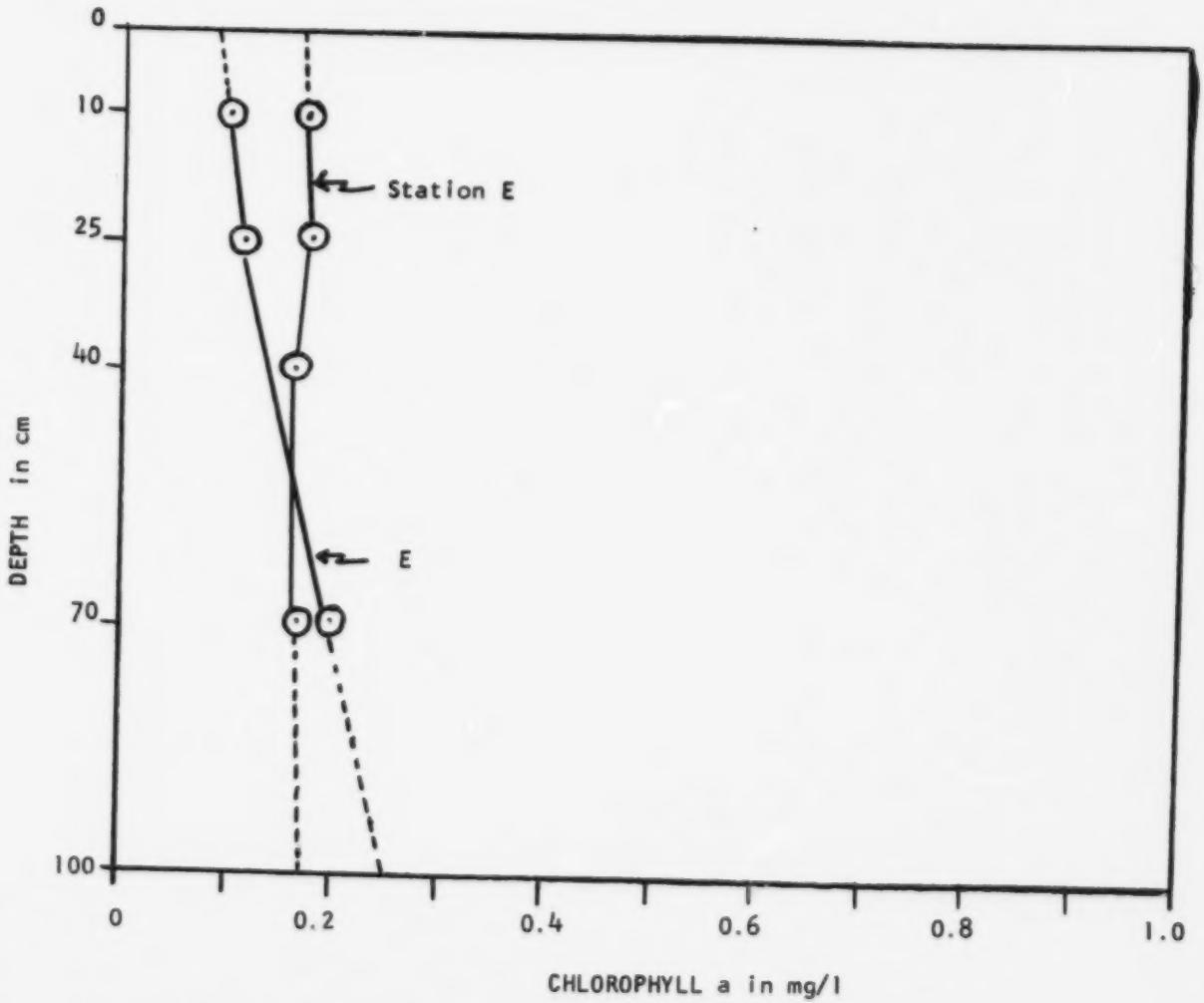

Figure 9. Chlorophyll a concentration in relation to depth at station E in August 1960.

0.5 ly/min (0.5 gram Cal/Cm^2/ min) or less, and the Chlorophyll a concentrations were less than one gram /M^3. In many instances when light intensity was low at the time of sample collection, this low intensity was due to fog or clouds rather than to the hour of the day. At surface light intensities above 0.5 ly/min and with Chlorophyll a concentrations greater than one gram /M^3, the vertical distribution of Chlorophyll a was not uniform. Variations in the depth profile of Chlorophyll a in relation to surface light intensity appear to be independent of the season of the year.

In Figures 4 and 5 the quantities of Chlorophyll a observed at specific depths have been plotted against observation time to form smooth curves. The concentrations of Chlorophyll a at pond depths of 10 and 40 on were found to vary with changes in light intensity at the surface of the pond. During the early morning hours, the rate of increase in the concentrations of Chlorophyll a at the different sampled depths were about the same; however, after 1000 hours the concentration of Chlorophyll a was increasing in abundance more rapidly at 40 than at 10 cm. (Figure 4). Late in the afternoon, the concentrations of Chlorophyll a were still increasing at 10 cm, but were decreasing at 40 cm.

In Figure 10 the concentrations of Chlorophyll a at each of the sampled depths at station E were plotted as a continuous series in relations to time of day. This figure shows the variability in the vertical distribution of Chlorophyll a as it occurred throughout a daytime. Figure 11 schematically represents the vertical distribution of Chlorophyll a at discrete times during the day at station E. Depth profiles, such as Figure 11, illustrate clearly the vertical distribution of chlorophyll at the time of sample collection. Since Figures 10 and 11 represent the same data, a comparison of these figures illustrates not only the way Chlorophyll a was vertically distributed at the time of sample collection but the mode of change in these vertical distributions in relation to a changing surface light intensity.

Daily Variations in the Mean Concentration of Pigments per Cubic Meter of Pond Water.

Using the depth profiles of pigment concentrations observed at four sampled depths (e.g. figures 6-9), planimetry was employed to estimate the mean concentration of pigment per cubic meter in a sampled column of water. Estimates of pigment concentrations so obtained are hereafter referred to as the "mean" concentration of a pigment. The mean concentration of Chlorophyll a/M^3 and Non-astacin pigments /M^3 observed at station E on July 19 and July 20, 1961 have been plotted against the time of day in Figures 13 and 14, respectively. One June 21 (Figure 12) the mean concentrations of Chlorophyll a were estimated from samples collected at two depths, 10 and 40 cm, in the pond. The concentrations of Chlorophyll a and Non-astacin pigments observed over a 26 hour period at the outfall of the pond on July 22 have been graphed in Figure 15, however, these latter concentrations represent individual samples taken from water flowing over the weir rather than mean con-

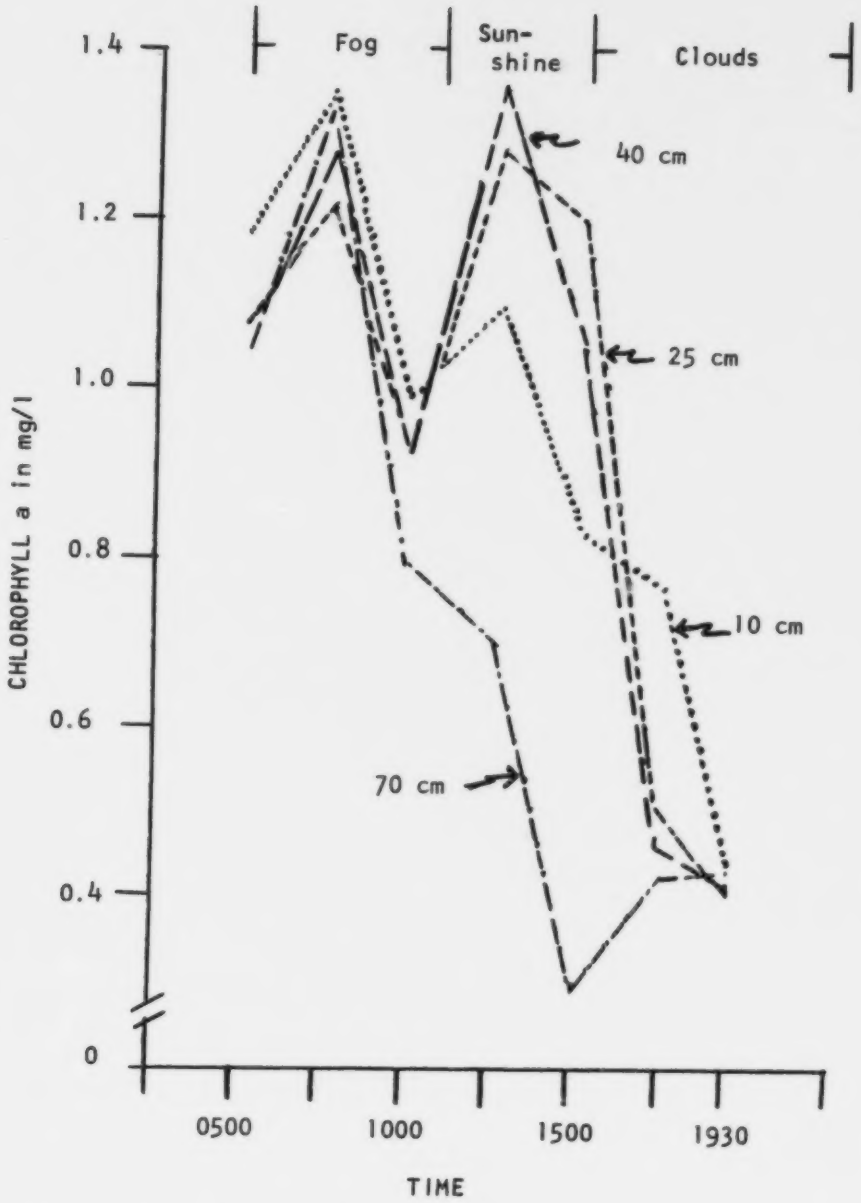

Figure 10. The concentrations of Chlorophyll a at depths of 10, 25, 40, and 70 cm at different times of day on July 19, 1961.

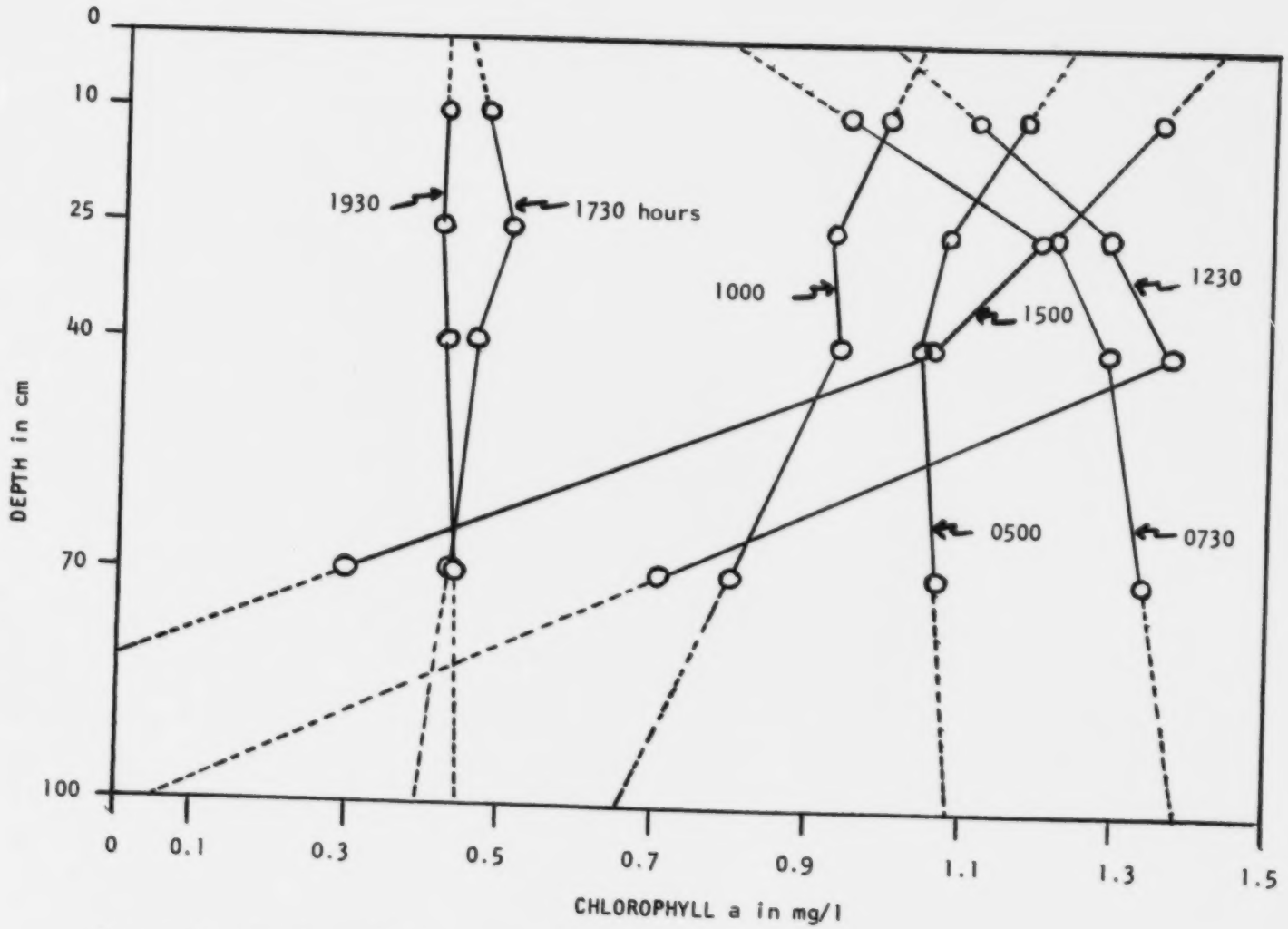

Figure 11. Depth profiles of Chlorophyll a concentrations at different times of day on July 19, 1961 at station E.

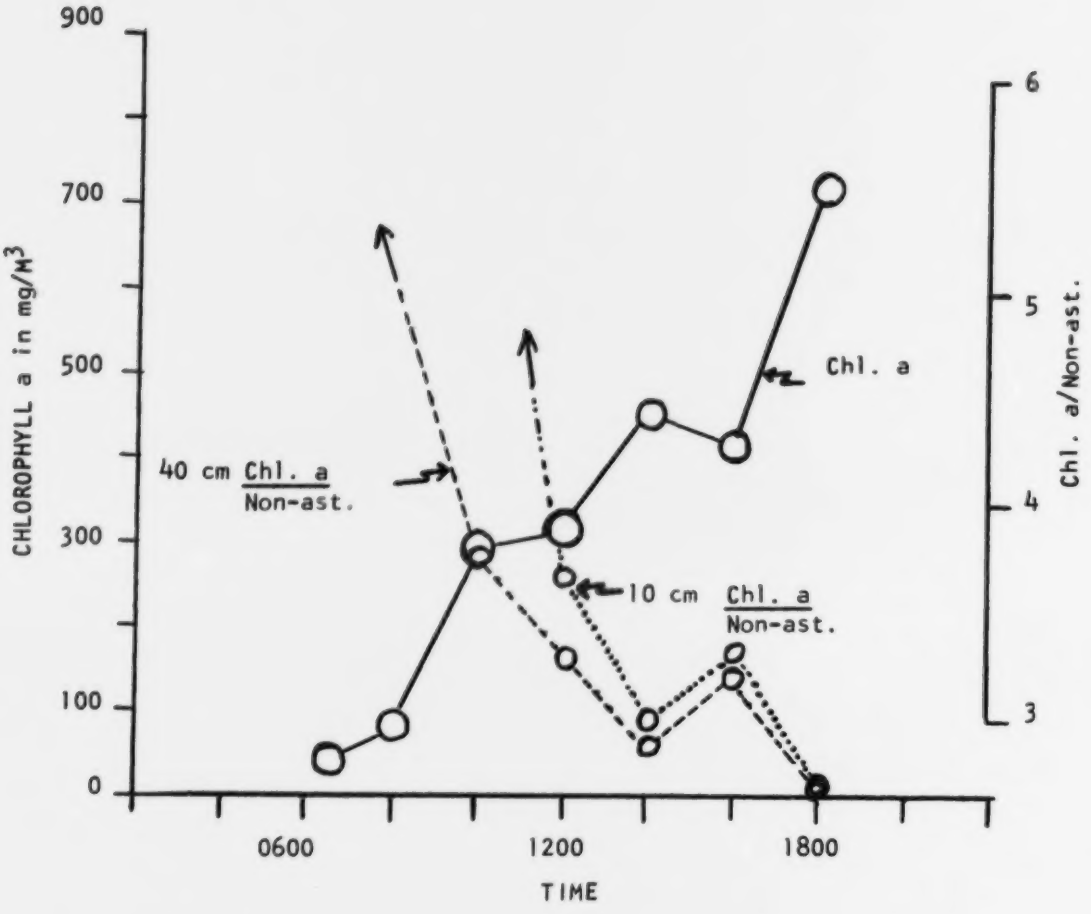

Figure 12. The mean concentration of Chlorophyll a per cubic meter in relation to the Chl. a/Non-ast. ratios at depths of 10 and 40 cm at different times of day on June 21, 1961.

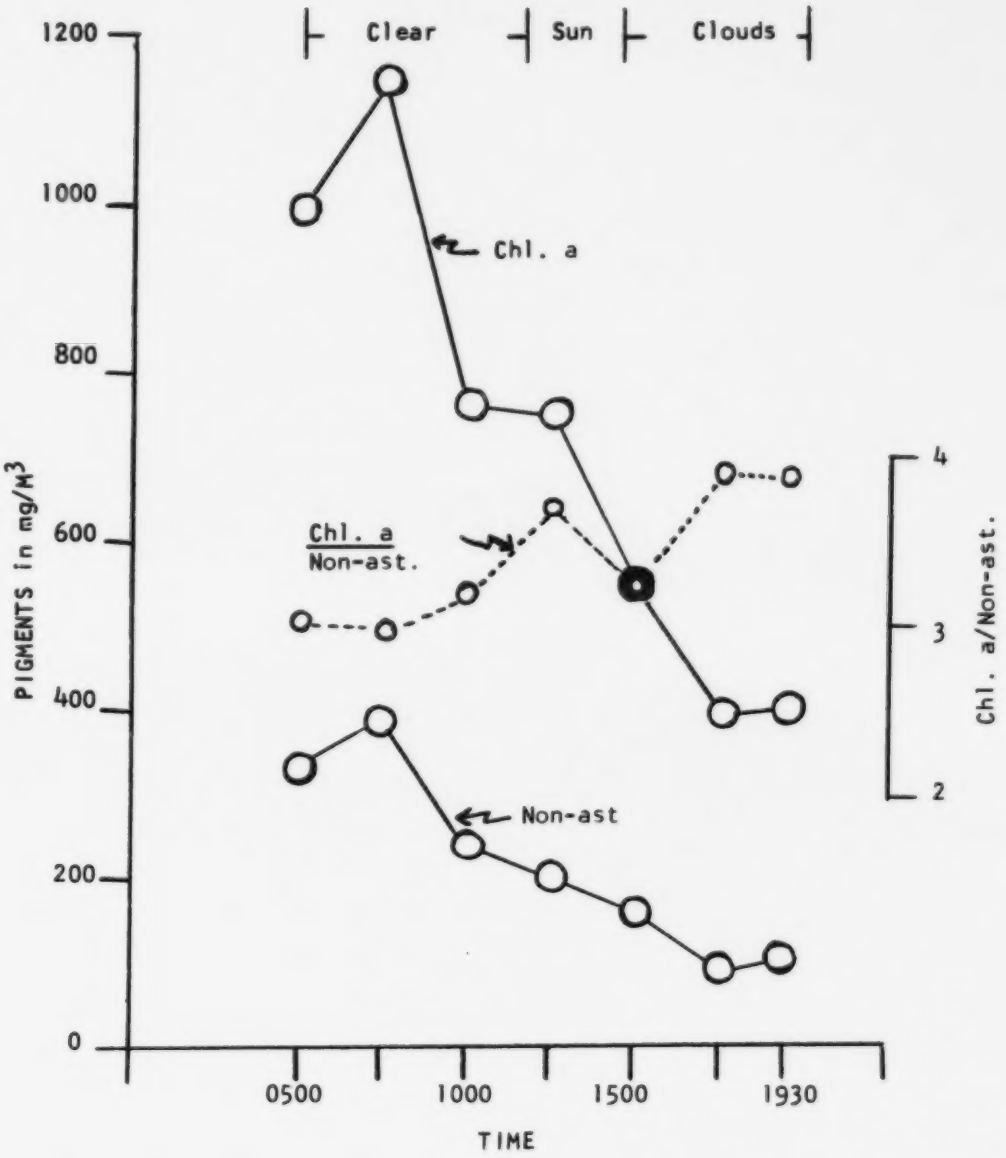

Figure 13. Chlorophyll a in mg/l and the Non-astacin pigments in MSPU/l as determined from samples collected at four depths with the corresponding Chl. a/Non-ast. pigment ratio at different times of day on July 19, 1961.

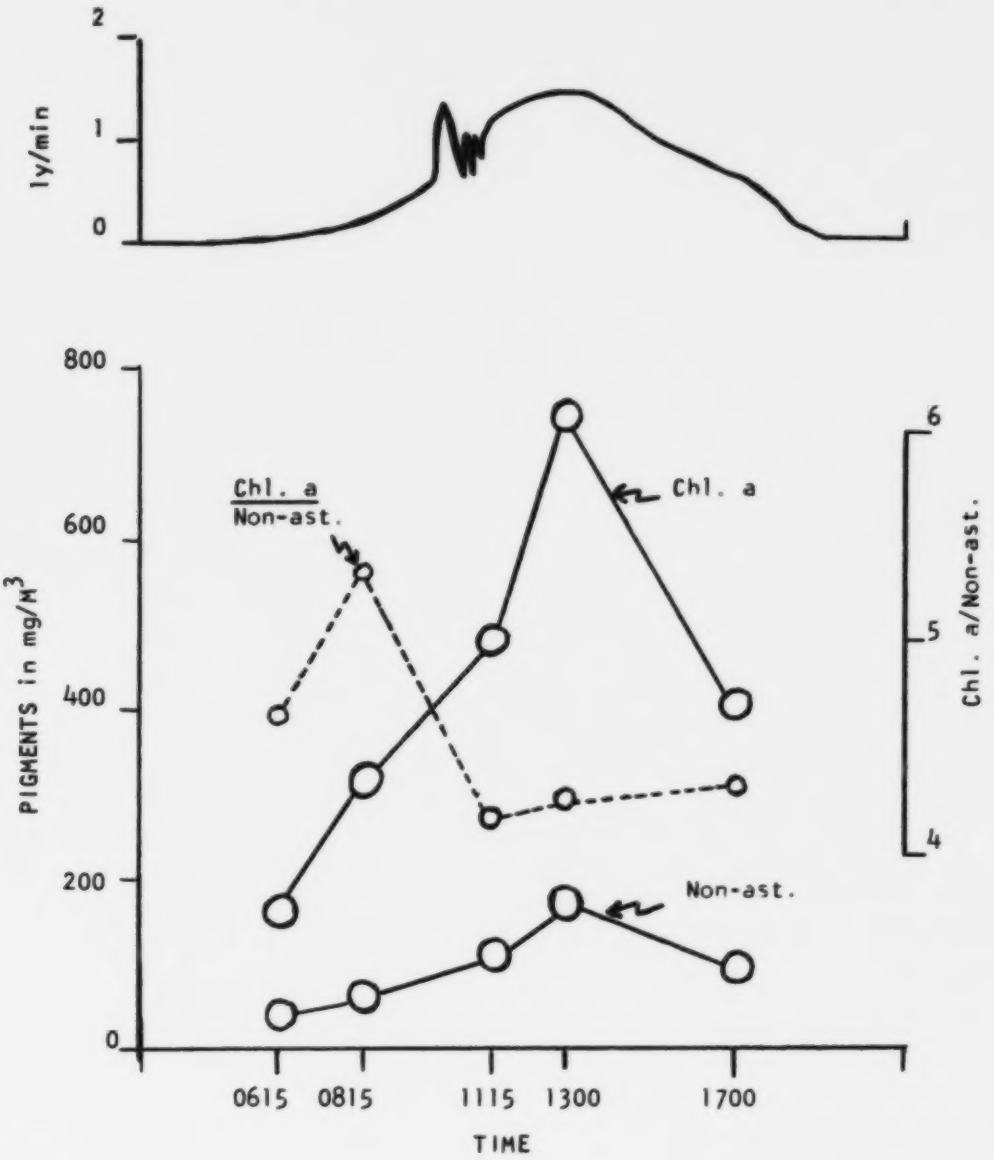

Figure 14. Chlorophyll a in mg/l and Non-astacin pigments in MSPU/l as determined from samples collected at four depths with the corresponding Chl. a/Non-ast. pigment ratio at different times of day on July 28, 1961.

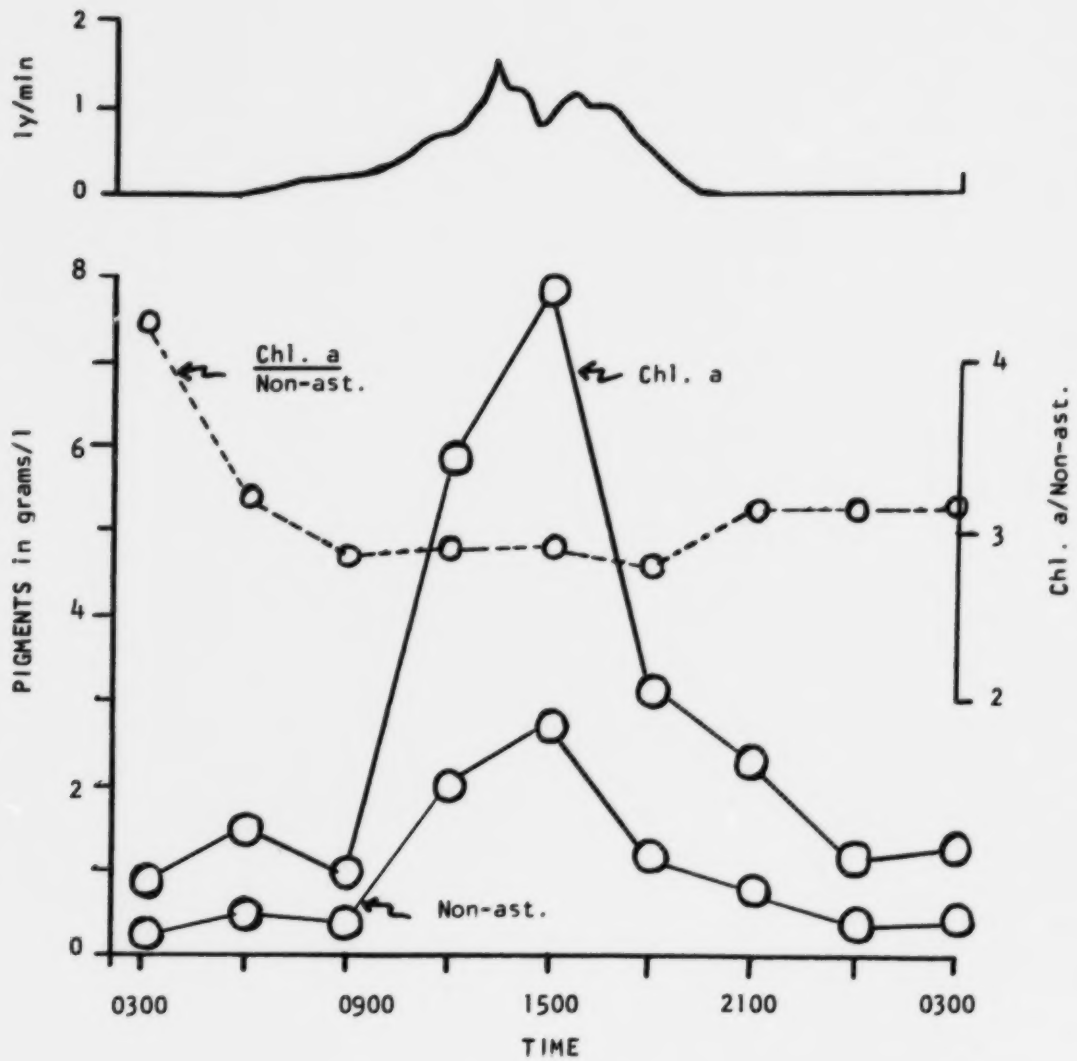

Figure 15. The concentrations of Chlorophyll a and Non-astacin pigments observed at the outfall during a 26 hour period on July 22 and 23, 1961.

On June 21 (Figure 12) there was fog until 1000 hours, but the remainder of the day was clear. During this day, there was a general increase in the mean concentration of chlorophyll a from about 40 mg/M^3 at 0615 hours to about 700 mg/M at 1800 hours. On July 19 (Figure 13) the sky was overcast except for a period of time between 1200 and 1400 hours when there was sunshine. During this day, there was a general decrease in the mean concentration of Chlorophyll a except for the period of time when there was direct sunlight. During this period of sunshine, there was not much change in the concentration of Chlorophyll a as opposed to a general decrease in the mean concentration for the complete day. A rapid change in the manner in which the concentrations of Chlorophyll a were vertically distributed compared to what was present during the time when the sky was overcast occurred during this period of sunshine (Figure 10). At 1230 hours the greatest concentration of Chlorophyll a, 1.4 mg/l, was observed at 40 cm and the least concentration, 0.7 ag/l, was at 70 cm although the average concentration for the water column was 0.75 mg/l.

When plotted against time, the mean concentrations of Chlorophyll a and Non-astacin pigments observed on July 28 (Figure 14) and July 22 (Figure 15) formed curves comparable in shape to the curves representing surface light intensity against time.

During the clear day of June 21, the mean concentration of Chlorophyll a increased by a factor greater than 10 or from 40 mg/M^3 to 700 mg/M^3. With a decreasing mean concentration of Chlorophyll a, such as occurred on July 19, the mean concentration was reduced by a factor of about 3. In all cases observed, if the mean concentration of Chlorophyll a for a complete day were based on only one sample, an error of several hundred per cent could result.

Daytime variations in the mean concentrations of Non-astacin pigments at station E were similar to those of Chlorophyll a, but the changes were not in a one to one ratio quantitatively.

Figure 16 illustrates the changes in proportion by percentage of the mean concentration of Chlorophyll a occurring in the upper 50 cm of the water column at station E on July 28, 1961. During this particular day, 50 to 80 per cent of the mean concentration of Chlorophyll a was observed in the upper 50 cm.

Horizontal Distribution of Chlorophyll a

Samples of phytoplankton were collected at stations D, E, J, and H within a one hour period at a depth of 25 cm on five different days. The concentrations of Chlorophyll a, as determined from these samples, are listed in Table I. Horizontal differences observed in the concentrations of Chlorophyll a between stations were quite large. The "relative difference" (Chl a-min/ Chl a-max) varied from about 0.32 to 0.83.

Water masses in the pond moved quite rapidly when there was a strong

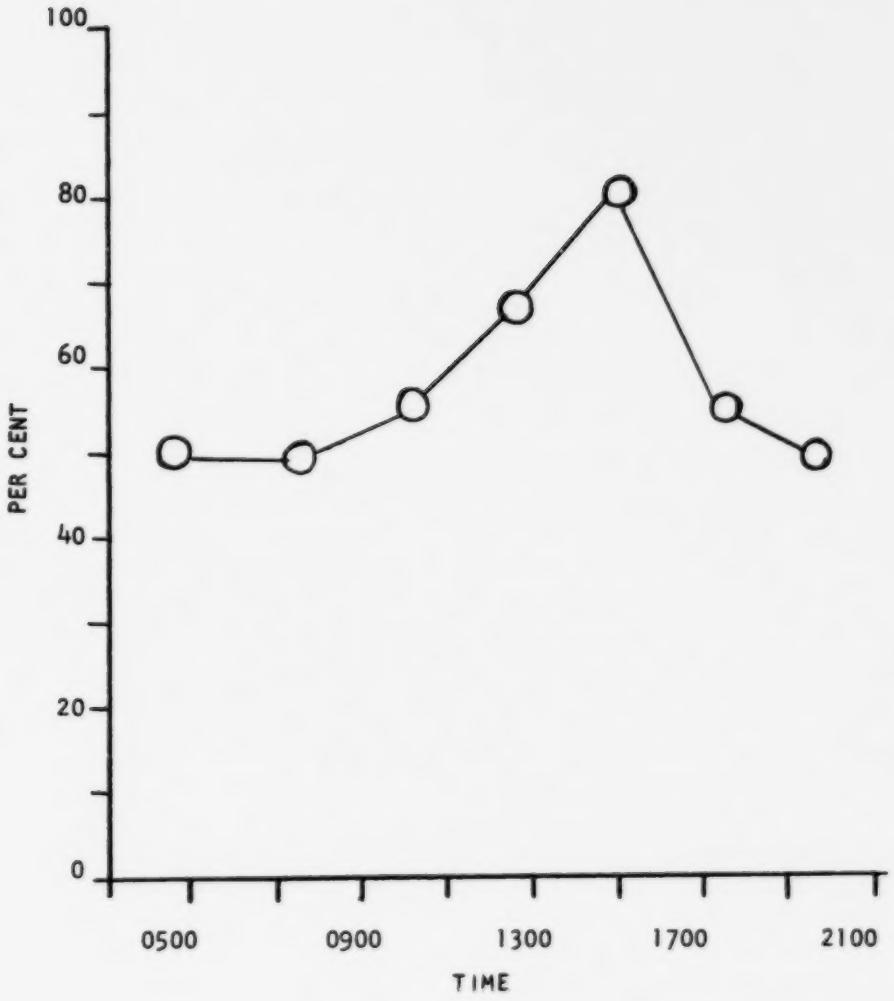

Figure 16. The percentages of the mean concentration of Chlorophyll a per cubic meter found in the upper 50 cm of the water column at different times of day on July 28, 1961.

wind. Resulting from these horizontal movements of a water mass, it is conceivable that the concentrations of Chlorophyll a observed over a period of hours at a stationary station may have been influenced by variations in the horizontal distribution of such chlorophyll. The manner in which these variations in horizontal distribution influenced the reported results from such a station was not evaluated.

Table 1 The concentrations of Chlorophyll a in mg/M^3 sampled at 25 cm depths at four stations in the pond during the same one hour period on each collection date.

Date	Stations				$\frac{\text{Chl. a-min}}{\text{Chl. a-max}}$
	D	E	H	J	
8-1-60	103	319	278	222	0.32
8-18-60	551	714	545	586	0.77
8-30-60	104	126	130	113	0.83
3-31-61	179	267	303	319	0.56
6-9-61	1188	1052	2155	1357	0.50

Chlorophyll a: Non-astacin Pigment Ratio

The Non-astacin (carotenoid) pigments of phytoplankton are those pigments other than chlorophyll. Parsons (1961) and Ketchem (1958) found that there was a variable Chlorophyll a: carotenoid ratio: and Strickland (1960) suggests that the carotenoids may be a better index to the biomass of phytoplankton than Chlorophyll a. The relationship between short-term changes in concentrations of Chlorophyll a and Non-astacin pigments were investigated during this study. The daytime Chlorophyll a; Non-astacin pigment ratio (Chl. a/Non-ast.) at different times of day in the pond followed the general pattern illustrated in Figure 17. On clear days, the ratio was highest in the morning, lower and more nearly constant during mid-day, and increasing again in the early evening.

Figures 14 and 18 show depth profiles of Chlorophyll a, and Figures 17 and 19 show depth profiles of Non-astacin pigments for different times of day on July 22 and July 28, 1961, respectively. Although the

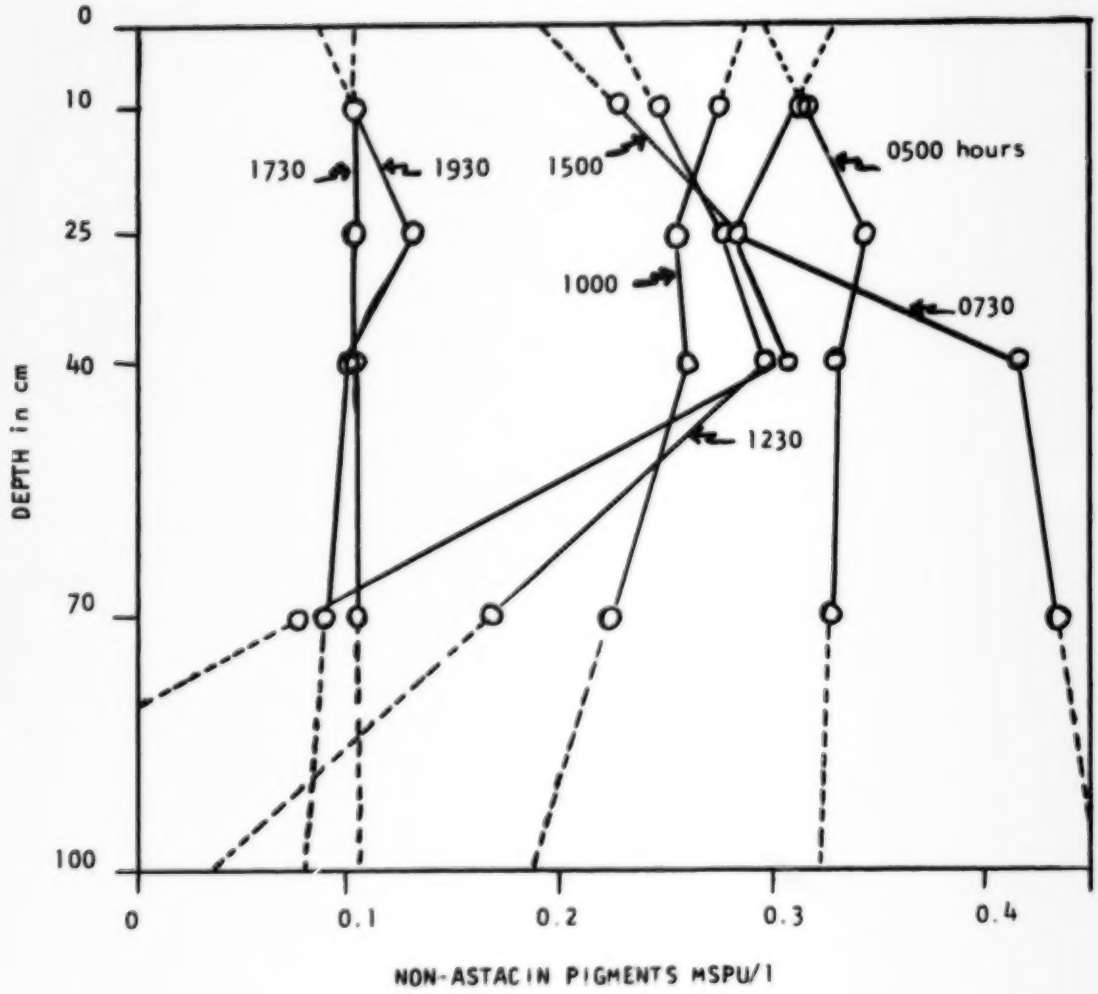

Figure 17. The depth profiles of the Non-astacin pigments at different times of day on July 19, 1961.

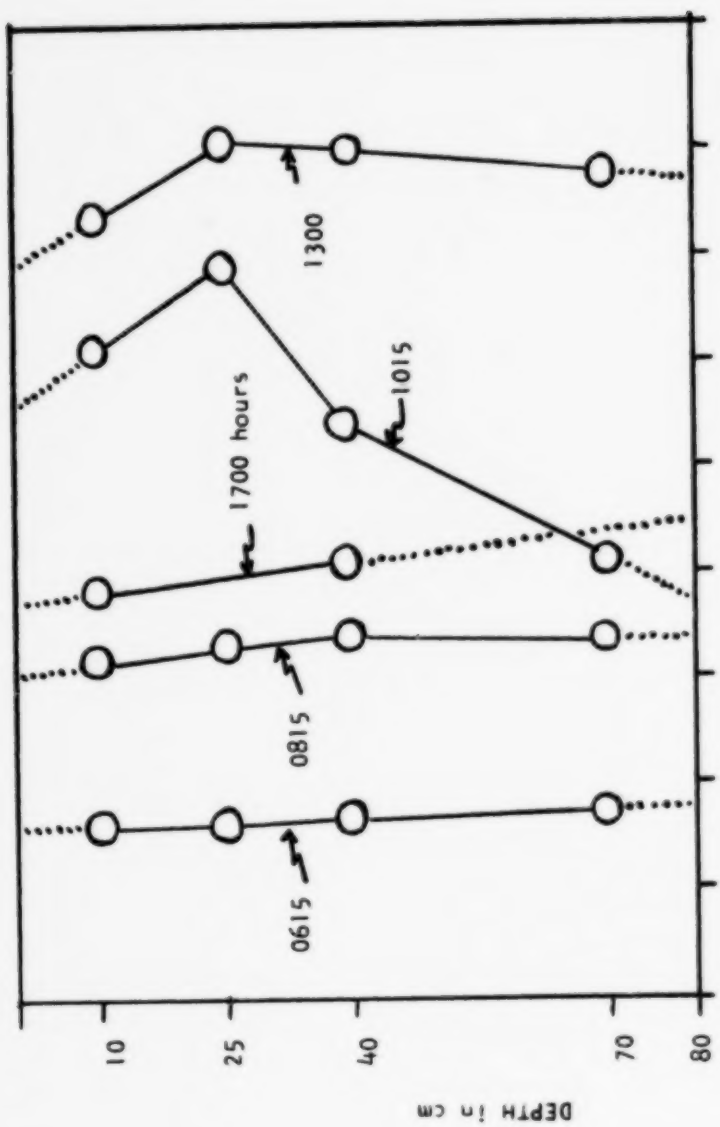

Figure 18. The depth profiles of Chlorophyll a at different times of day on July 28, 1961 at station E.

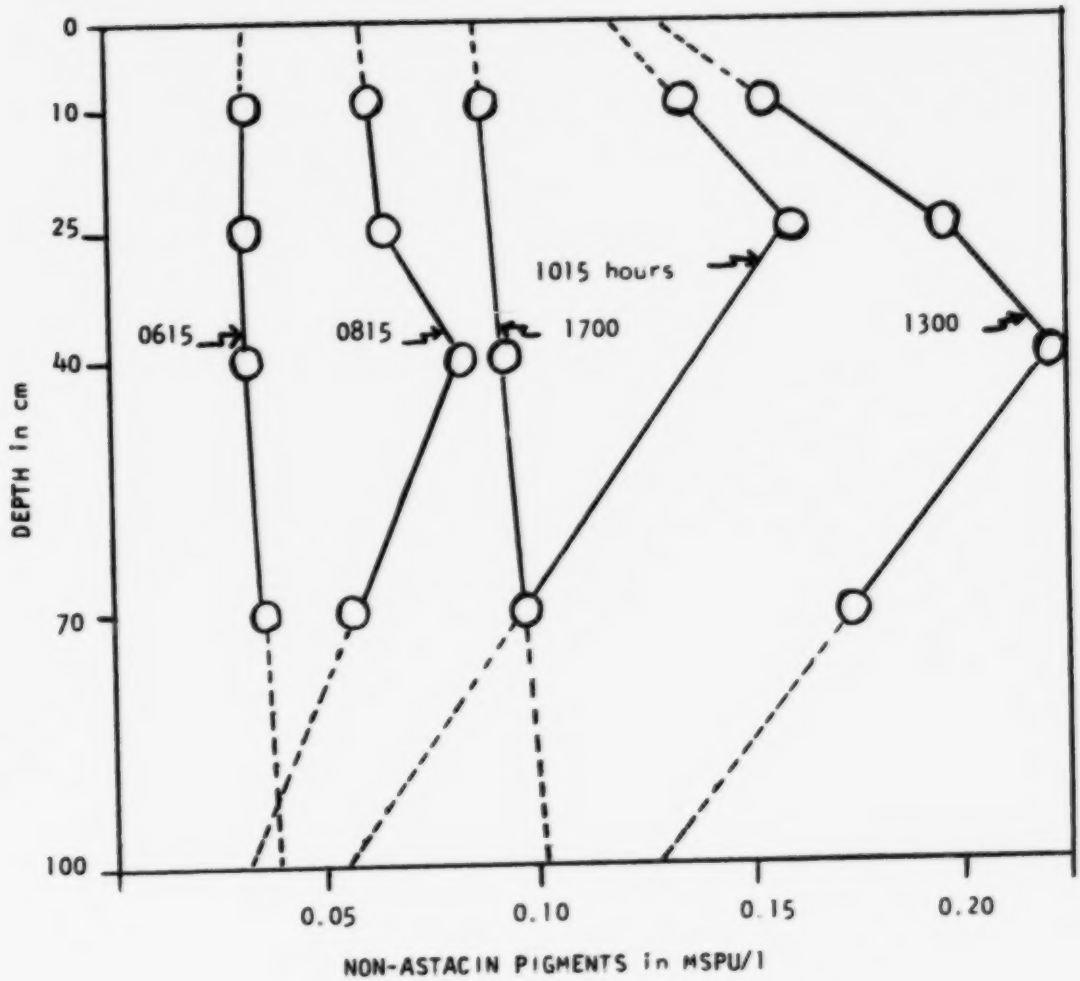

Figure 19. The depth profiles of Non-astacin pigments at different times of day on July 28, 1961.

depth profiles of both types of pigment generally have very similar shapes, there were some notable exceptions. For example, the shape of the depth profiles of the two types of pigments at 0815 and 1300 hours on July, 1961 (Figures 18 and 19) are quite different. The differences are best illustrated by comparing the Chl. a/Non-ast. ratios of these samples. At 0815 hours the ratios were 5.14, 5.08, 4.11, and 5.89 at 10, 25, 40, and 70 cm, respectively. In this case the greatest variation in concentration between the two pigments was at a depth of 40 cm. At 1300 hours the ratios were 4.82, 4.11, 3.61, and 4.47 at 10, 25, 40 and 70, cm, respectively, and again, the greatest variation was at a depth of 40 cm. By comparing the depth profiles for 0815 and 1300 hours in Figures 18 and 19, differences in shape, as indicated by variations, in the Chl. a/non-ast. ratios, are evident.

Another means of comparing differences in the concentrations of Chlorophyll a and Non-astacin pigments in the same samples is shown in Figure 20. In this figure the concentrations of Chlorophyll and Non-astacin pigments at station E on July 19, 1961 (depth profiles in Figures 14 and 18) were compared directly. The concentrations of the two types of pigments were plotted as relative concentrations (Pigment-observed/Pigment-maximum X 100) for each set of samples in relation to the time of collection. From these depth profiles of the relative concentrations of the two types of pigments, it is apparent that variations in concentration between the two types of pigments do not occur at the same rate throughout the water column.

In Figures 21 through 24 the concentrations of Chlorophyll a at depths of 10, 25, 40, and 70 cm at station E on July 19, 1961 were plotted against the Chl. a/Non-ast. ratio for each sampled depth. The relationship between the concentration of Chlorophyll a/l and the Chl. a/Non-ast. ratio appears to be direct at a depth of 10 cm in that with an increase in the concentration of Chlorophyll a there was a corresponding increase in the Chl. a/Non-ast. ratio. The same general relationship was apparent for samples obtained at a depth of 25 cm, but did not apply to samples taken at depths of 40 and 70 cm. The range in value of the Chl. a/Non-ast. ratio at depths of 10 and 25 cm was 3.03 to 4.65, and 3.08 to 4.82 for 40 and 70 cm depths. A greater range in the ratio was found when the upper 50 cm and the lower 50 cm of the water column were considered separately. The observed range was from 3.45 to 4.60 in the upper 50 cm and from 3.10 to 5.05 in the lower 50 cm. During the daytime until 1700 hours at station E on July 19, 1961, the Chl. a/Non-ast. ratio was larger in the upper 50 cm than in the lower 50 cm of the water column. After 1700 hours the reverse situation was observed (Figure 25).

Mean concentrations of Non-astacin pigments per cubic meter were estimated in the same manner as were the mean concentrations of Chlorophyll a from depth profiles (e.g. Figure 17) using planimetry. The observed mean concentrations of Non-astacin pigments a/M^3 for July 19, and July 28, 1961 were plotted against time of collectionin Figures 13 and 14, respectively. Mean Chl. a/Non-ast. ratios were calculated from the mean concen-

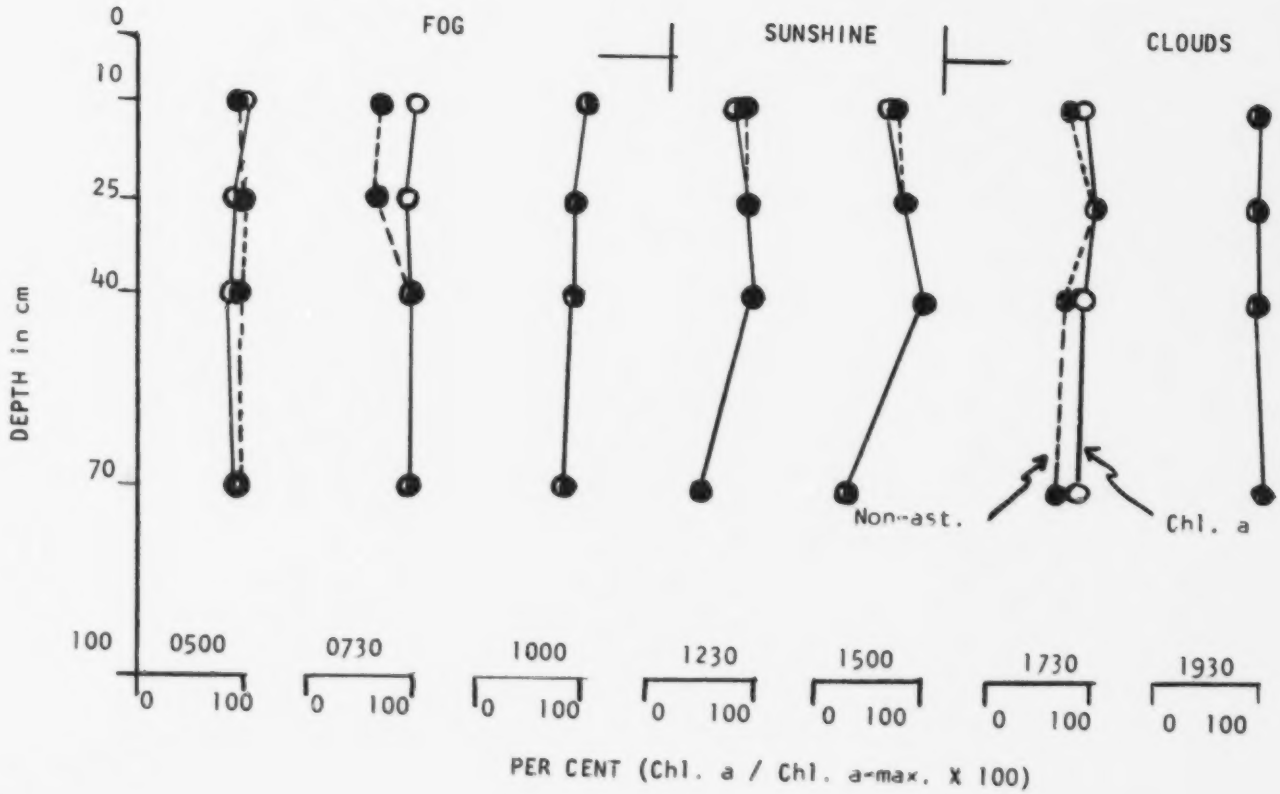

Figure 20. The per cent of the maximum occurring concentration of Chlorophyll a and Non-astacin pigments in each set of samples in relation to depth at different times of day on July 19, 1961.

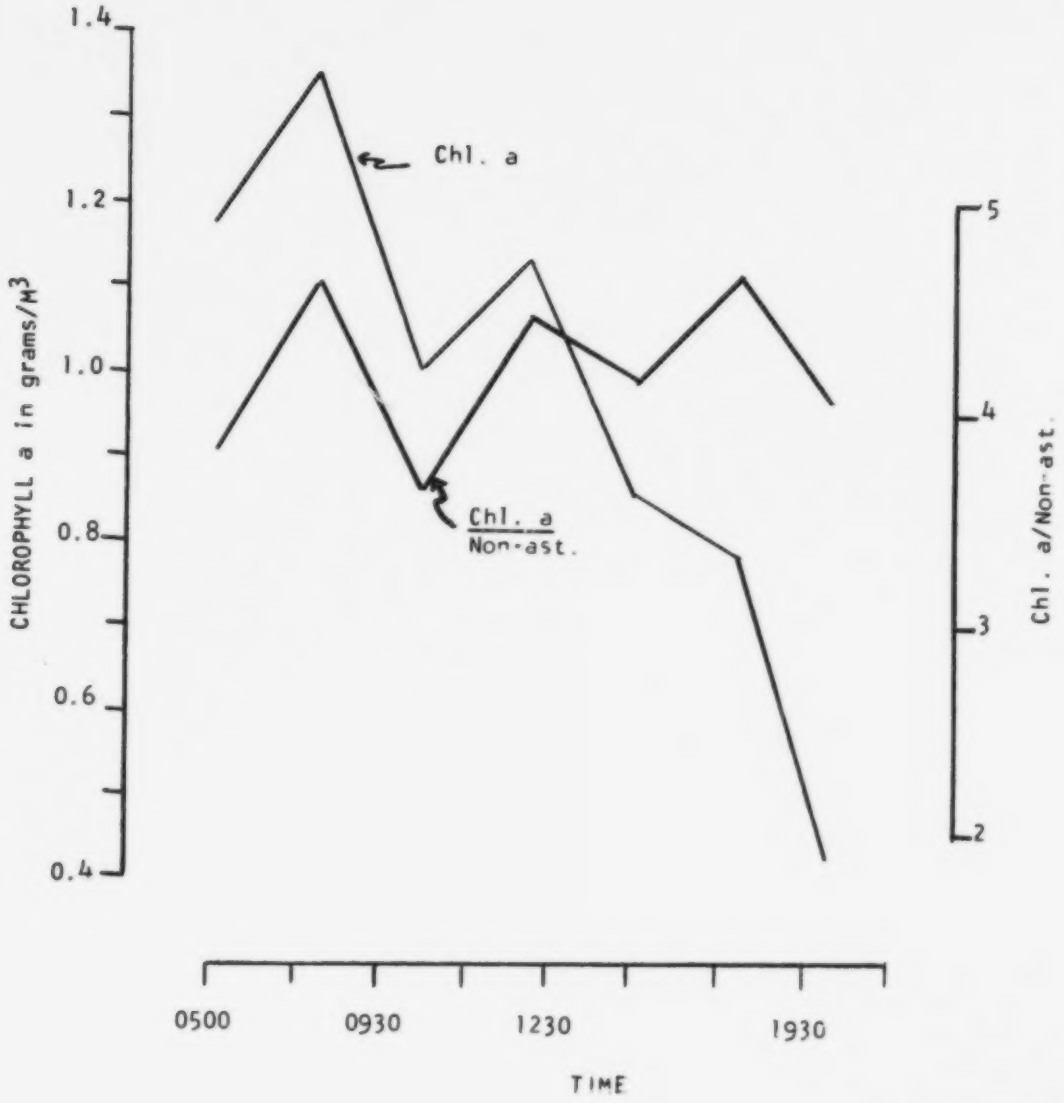

Figure 21. The relationship between the concentration of Chlorophyll a and the Chl. a/Non-ast. ratio at a depth of 10 cm at different times of day on July 19, 1961.

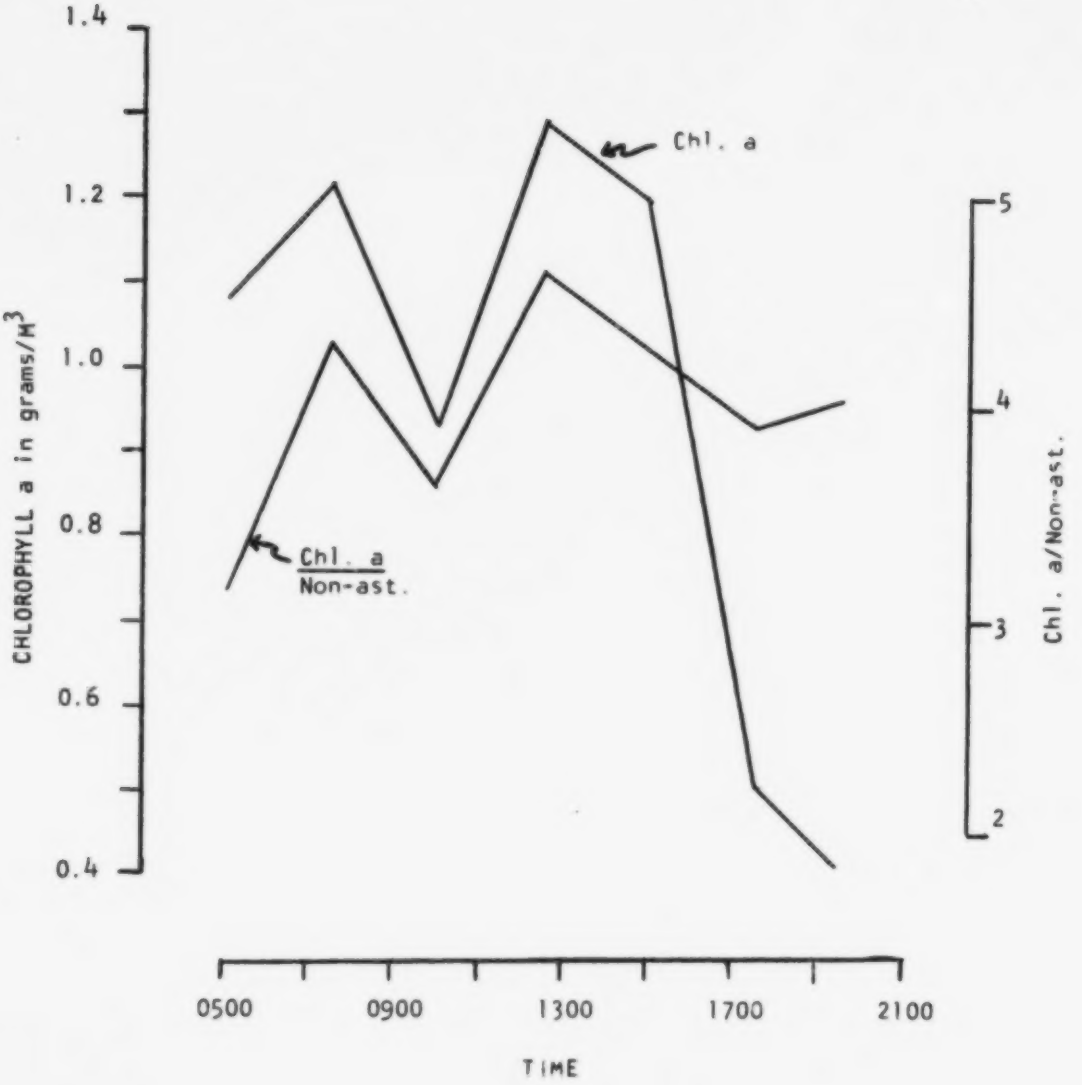

Figure 22. The relationship between the concentration of Chlorophyll a and the Chl. a/Non-ast. ratio at a depth of 25 cm at different times of day on July 19, 1961.

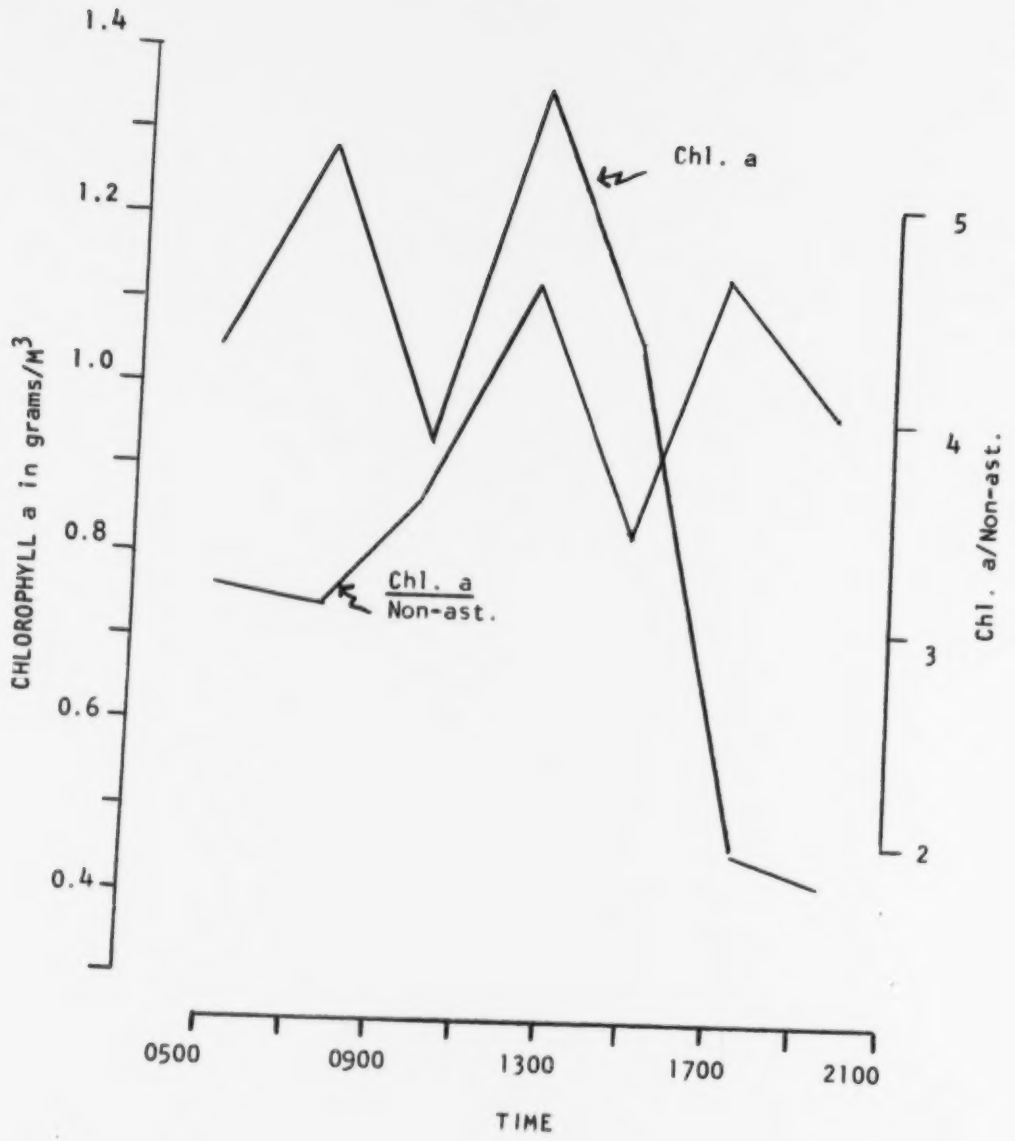

Figure 23. The relationship between the concentration of Chlorophyll a and the Chl. a/Non-ast. ratio at a depth of 40 cm at different times of day on July 19, 1961.

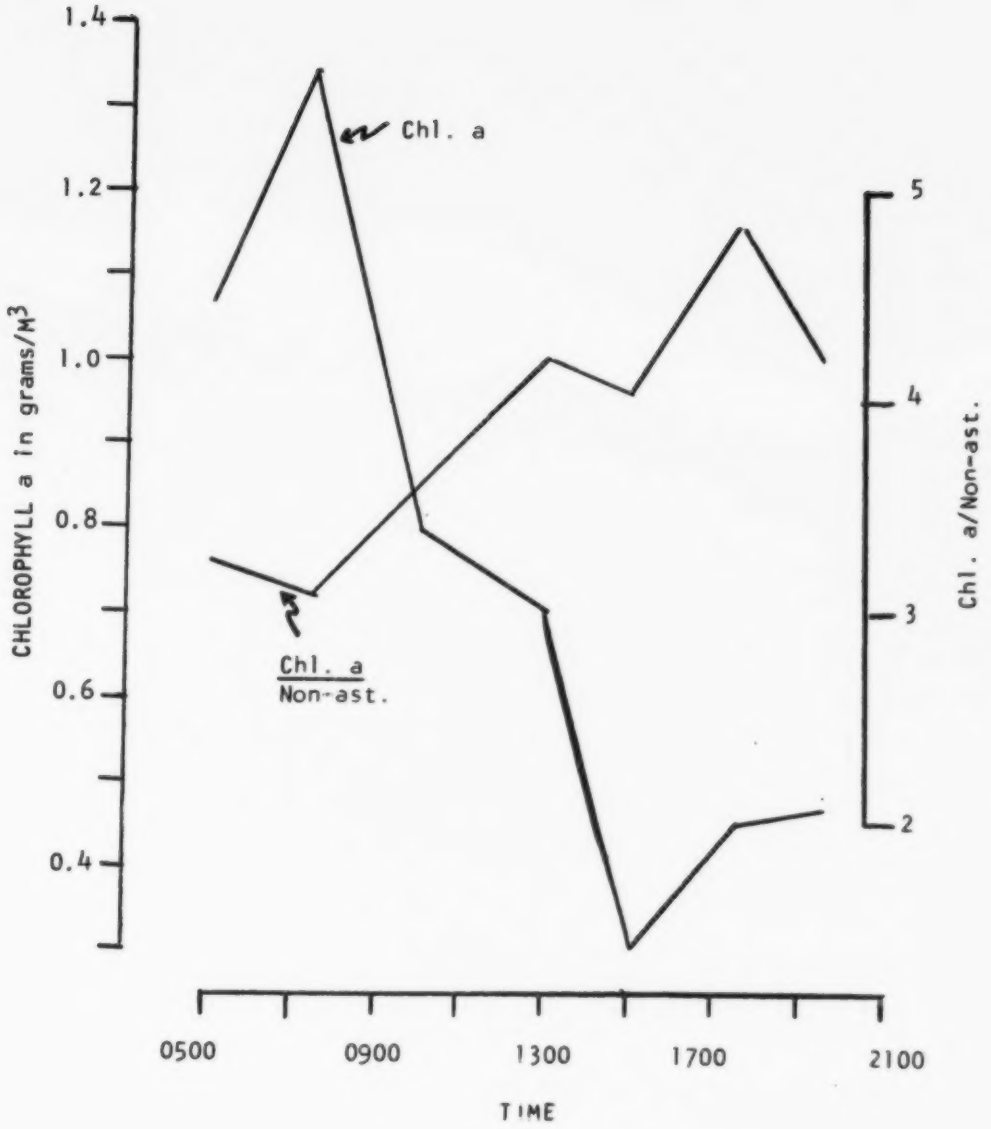

Figure 24. The relationship between the concentration of Chlorophyll a and the Chl. a/Non-ast. ratio at a depth of 70 cm at different times of day on July 19, 1961.

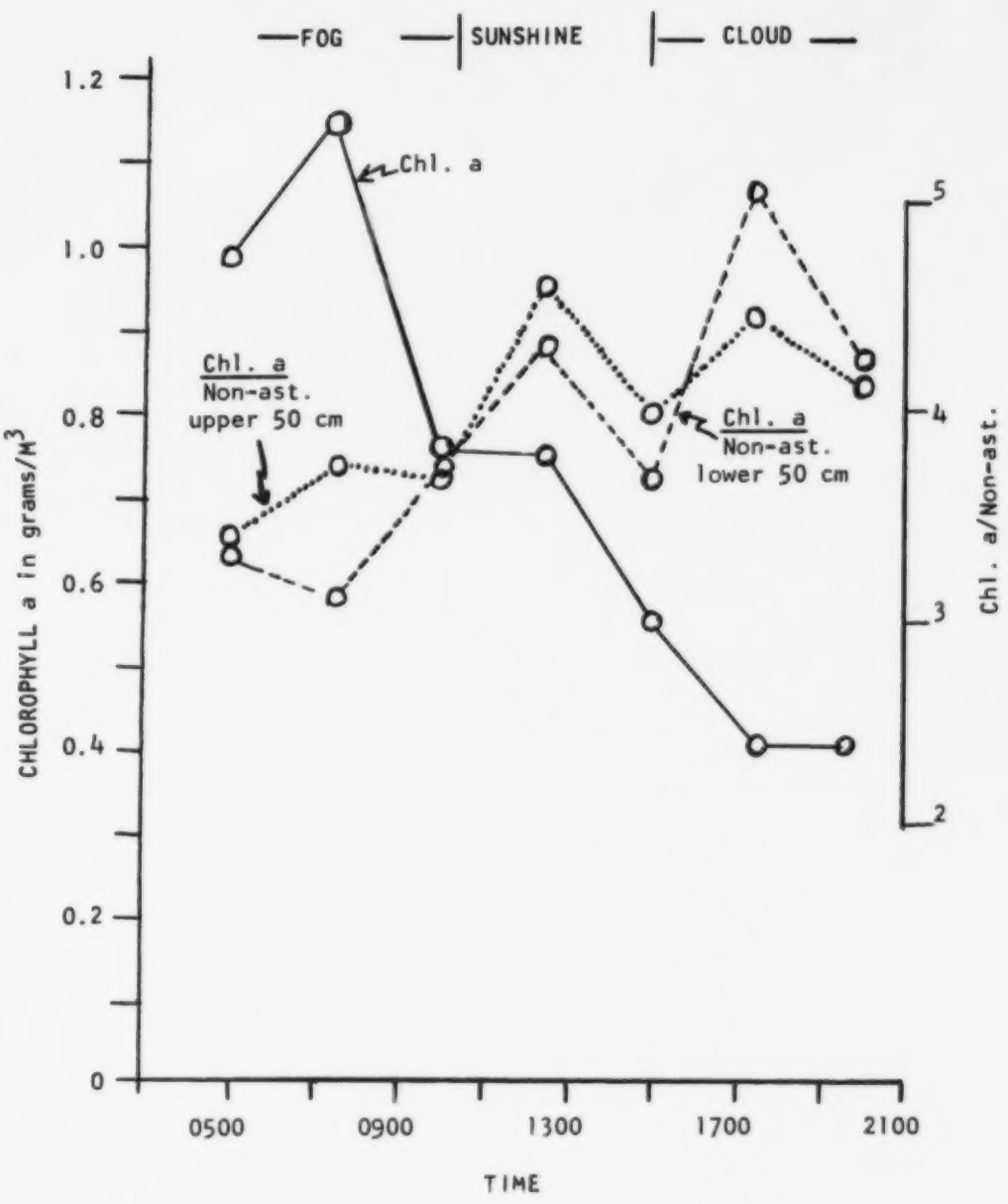

Figure 25. The concentration of Chlorophyll a/M^3 in relation to the Chl. a/Non-ast. ratios of the upper and lower 50 cm of the water column at different times of day on July 19, 1961.

trations of Chlorophyll a and Non-astacin pigments per cubic meter of water. These ratios varied from 2.5 to 4.5 (Figures 13 and 14).

As illustrated in Figures 21 and 22, the Chl. a/Non-ast. ratio at depths of 10 and 25 cm at station E on July 19, 1961 varied directly with changes in the concentration of Chlorophyll a at each depth. Each time there was an increase in the concentration of Chlorophyll a there was also an increase in the Chl. a/Non-ast. ratio, and vice versa. When mean Chl. a/Non-ast. ratios were compared to mean concentrations of Chlorophyll a, the general tendency was a decreasing mean ratio in relation to an increasing mean concentration of Chlorophyll a. Such results are shown in Figures 13, 14 and 15. This tendency is most clearly indicated in Figures 15 which shows both increasing and decreasing mean concentrations of Chlorophyll a on the same day.

Relationship Between the Concentration of Chlorophyll and Daphnia

From June through October 1960, Daphnia Magna and Chlorophyll a samples were collected from the pond. To estimate the average number of Daphnia per liter in the pond on a sampled day, two samples were collected at each of nine stations. The average number of Daphnia per liter per day was computed from these 13 samples. The amount of chlorophyll in the pond at the time of Daphnia sampling was usually based on a single sample of water collected at station E, but at times samples were collected at more than one station, and an arithmetic mean concentration of chlorophyll was computed. The number of Daphnia per liter and the concentration of Chlorophyll a per cubic meter observed was plotted against the time of collection. Curves were fitted to the plotted points in Figure 26 to indicate the fluctuations in concentrations of Daphnia and phytoplankton.

The abundance of Daphnia and phytoplankton (as indicated by Chlorophyll a concentrations) was cyclic and apparently related in a "predator-prey" manner. When maximum numbers (300 to 400 per liter) of Daphnia were present the concentration of Chlorophyll a was near zero, and at times when there were few or no Daphnia, the concentrations of chlorophyll a were greatest. These relationships were most apparent, during June and August 1960, when the two largest populations observed occurred. However, the same general relationships appeared to exist during other periods of time, but they were less obvious. Fluctuations in the number of Daphnia in the Arcata pond are believed due largely to fluctuations in the abundance of phytoplankton. Conversely, since Daphnia decimate the population of phytoplankton through "grating," fluctuations in the concentration of Chlorophyll a may at times be partly due to fluctuations in the abundance of Daphnia. By reducing the phytoplankton to very low levels, large populations of Daphnia were often indirectly responsible for the development of nearly anaerobic conditions in the pond. Further reductions in oxygen concentration were due directly, through respiration, to these large populations.

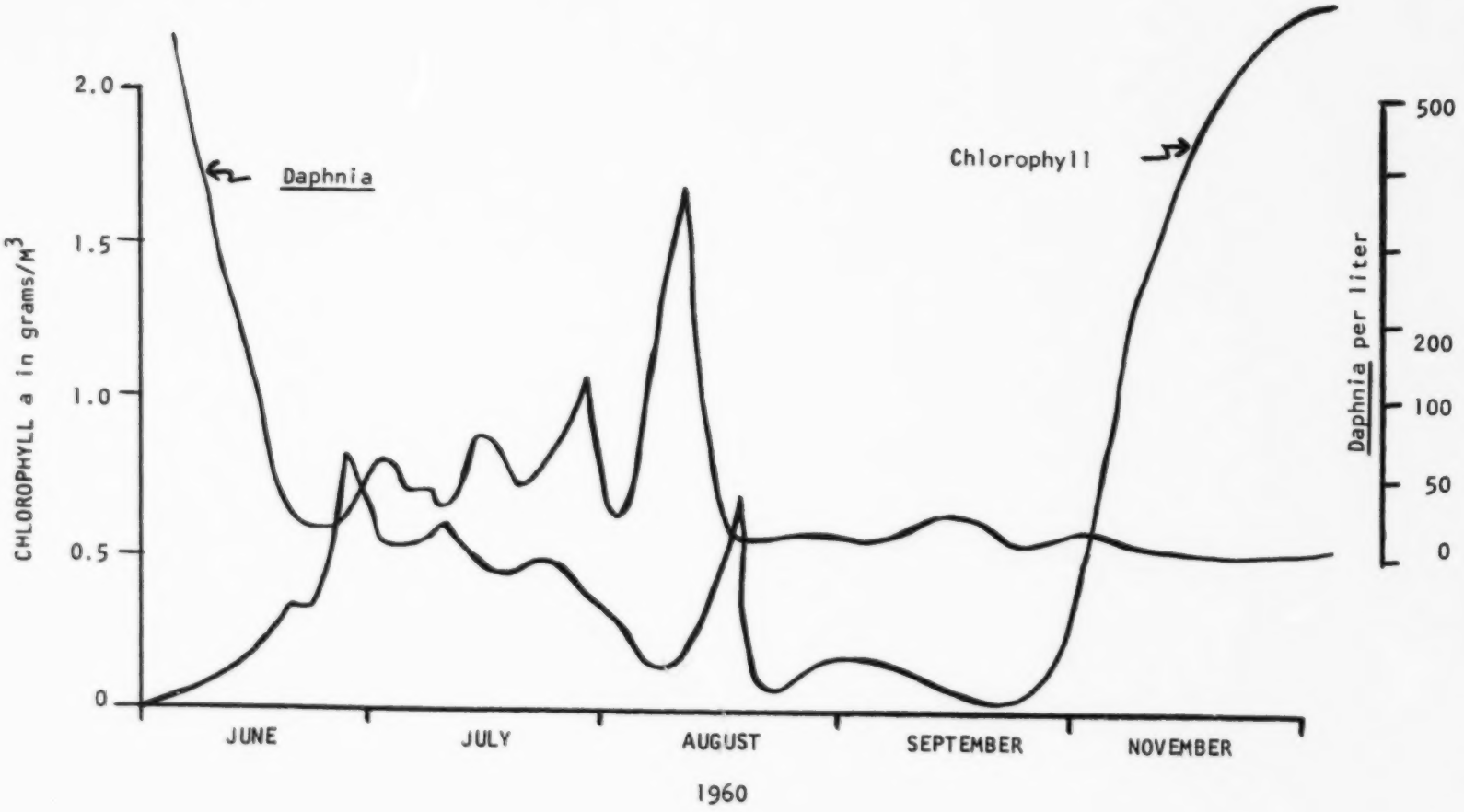

Figure 26. The relationship between the concentration of Chlorophyll a and Daphnia magna during the summer of 1960.

Concentrations of Chlorophyll a by Month

The observed mean concentration of Chlorophyll a in the pond during each of 11 months in 1960 and 1961 are indicated in Figure 27. Each mean is based on several samples taken each month at various stations, but always at a depth of approximately 25 cm. For days when more than one sample was obtained at any one station, the samples collected nearest 1100 hours were used for calculating mean concentrations of Chlorophyll a in the pond. Generally, the largest concentrations occurred during the seasons of the year with the highest light intensities. Although no fixed program of sampling was followed, the day when a sample was collected was determined to a large extent by the weather. Sampling was usually not done on stormy days, and in most cases, there were probably one or two clear or at least partly clear days preceding the day on which sampling was done. This procedure probably tended to cause sampling to be conducted on days when greater than truly average concentrations of Chlorophyll a prevailed.

Relationship Between Chlorophyll a and the Ash-free Dry Weight of Suspended Particulate Matter

The ash-free dry weight of suspended particulate matter and the concentration of Chlorophyll a were determined from duplicate samples collected between February and June, 1960. Samples were taken at stations D and I, (near the inflow) and at stations R and O (at the outfall). For purposes of analysis and reporting, samples from stations D and I and H and O were combined into "inflow" and "outfall" samples, respectively. Suspended solids for both ash-free dry weight and Chlorophyll a determinations were removed from the samples using a Foerst continuous centrifuge.

The ash-free dry weight of suspended matter is often used as an estimate of the amount of organic matter in water. Since phytoplankton contribute a significant amount of suspended organic matter to oxidation pond water, the relationship between ash-free dry weight and the concentration of Chlorophyll a was investigated to ascertain the relative amount phytoplankton contributed to the total amount of suspended organic matter. Manning and Juday (1941) found a chlorophyll ash-free dry weight ratio of 0.0013 to 0.0061 in some Wisconsin lakes. The ratio at the outfall of the Arcata pond was approximately 0.0013 to 0.0138. Wright (1959) at Canyon Ferry Reservoir, Colorado, found 1 microgram chlorophyll for each 0.12 milligram ash-free dry weight of suspended matter. At the Arcata pond outfall, 1 microgram of Chlorophyll a was equivalent to 0.07 to 0.18 milligram ash-free dry weight. The mean value was 0.09 milligram ash-free dry weight of suspended matter to 1 microgram Chlorophyll a.

Figures 28 and 29 illustrate the relationship between the concentration of Chlorophyll a and the ash-free dry weight of the suspended particulate matter from February through June 1960 near the outfall and inflow, respectively. A fairly high degree of correlation((correlation

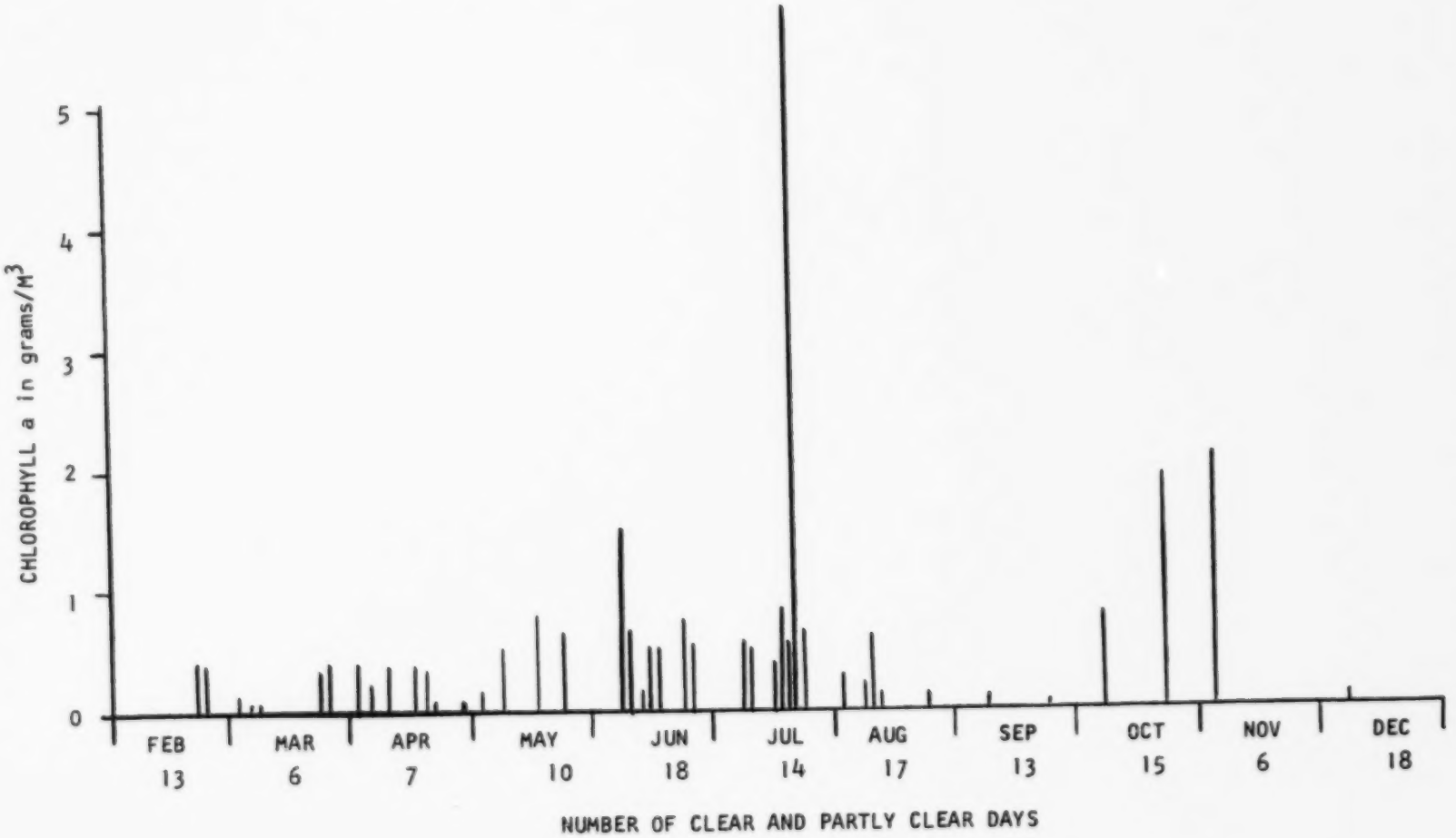

Figure 27. Concentrations of Chlorophyll a observed during 1960 and 1961. The number under each month designates the number of clear or only partly cloudy days during that month in 1960.

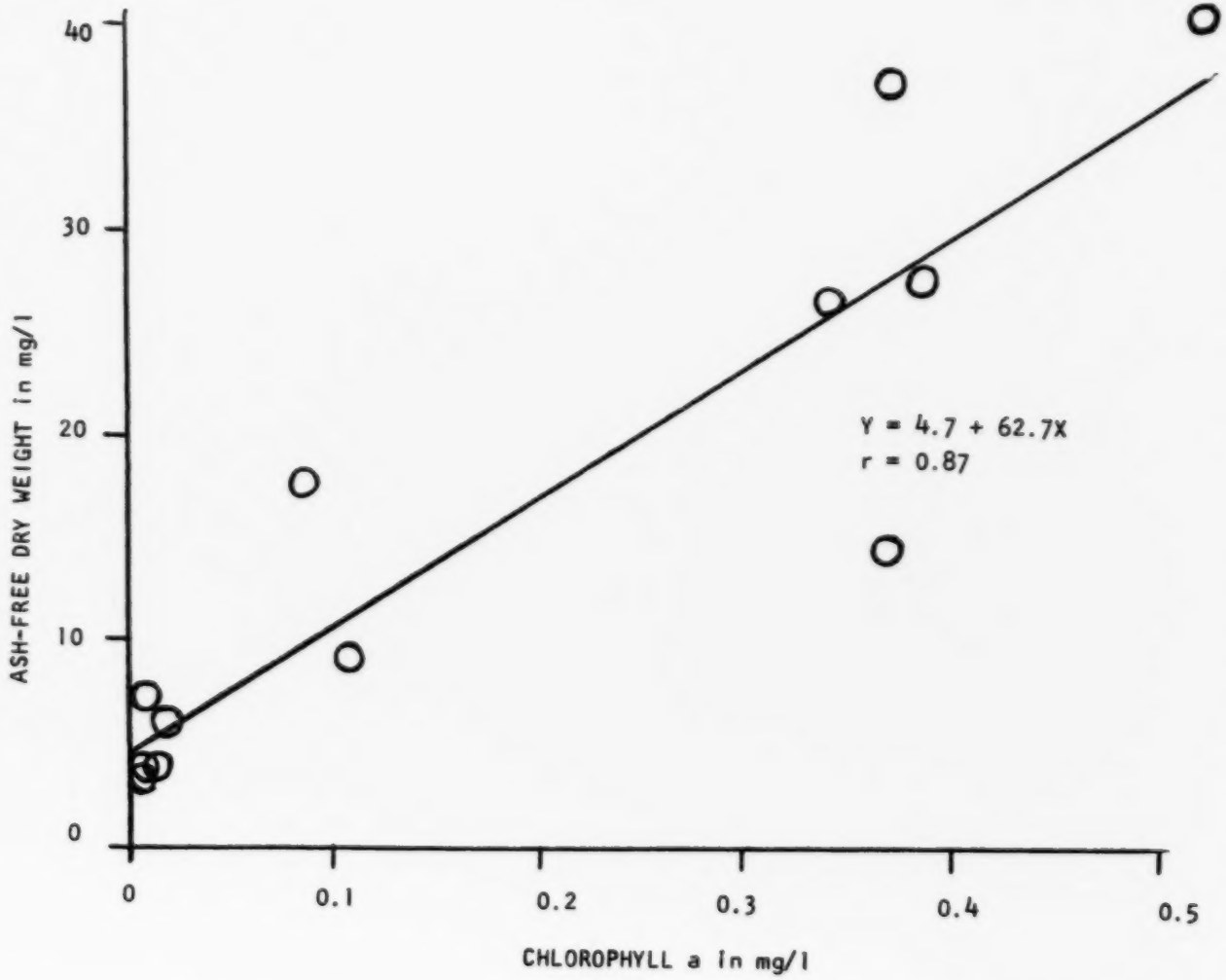

Figure 28. The relationship of Chlorophyll a concentration to the ash-free dry weight of the suspended solids at stations E and H during the spring of 1960.

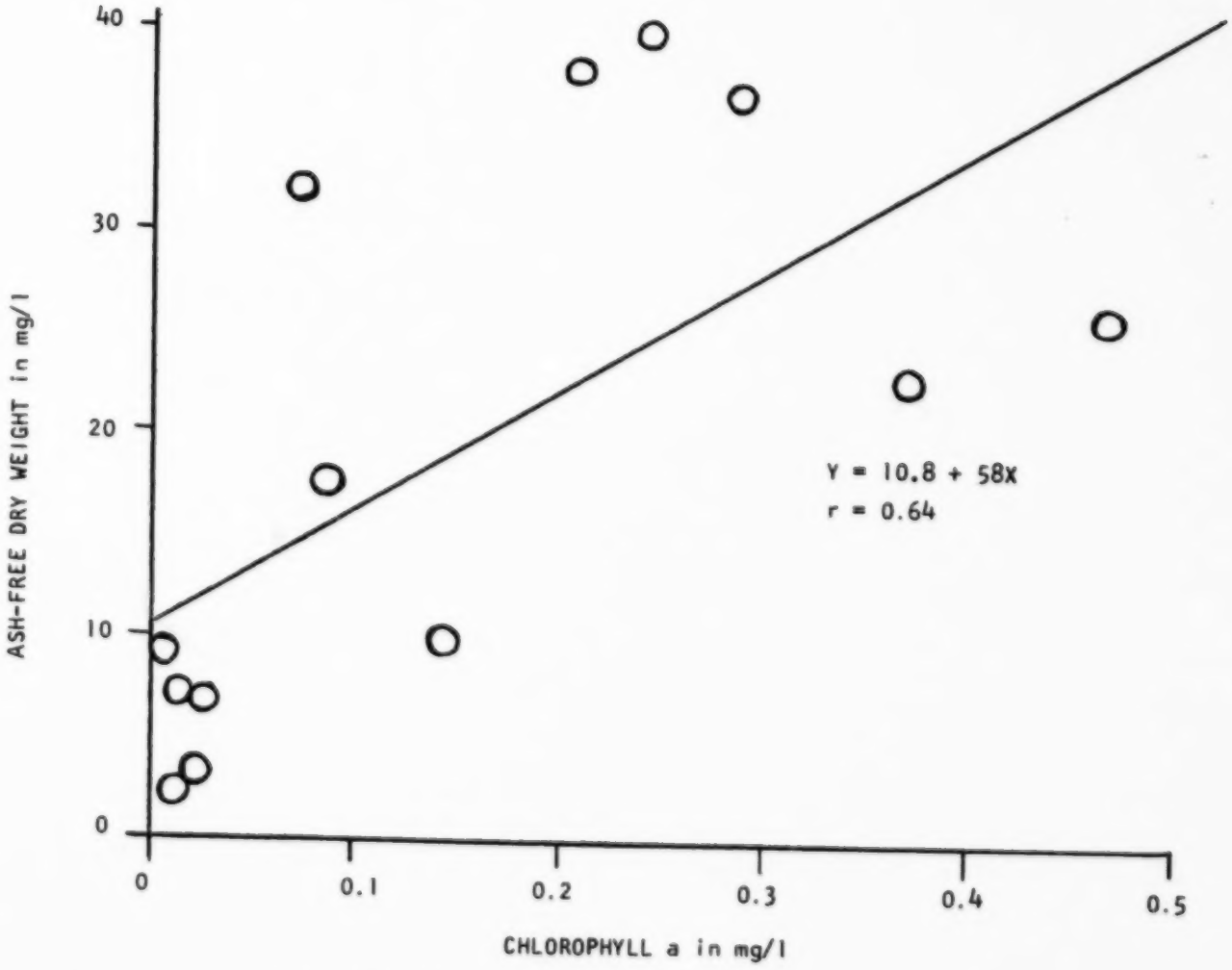

Figure 29. The relationship of Chlorophyll a concentration to the ash-free dry weight of the suspended solids at stations D and I.

coefficient, r=0.87) was found for the outfall samples, but there was a low degree of correlation (r=0.64) for the inflow samples. Seventy-six per cent (r^2=0.76) of the variation in organic weight per liter at the outfall perhaps can be attributed to changes in the concentration of Chlorophyll a, while only 41 per cent (r^2=0.41) of the variation in organic weight per liter perhaps was due to such changes in Chlorphyll a at the inflow.

RESULTS OF LIGHT AND DARK BOTTLE ANALYSIS

Gross Oxygen Production

Rhode (1958) reported on short-term determinations of primary productivity in a Swedish lake made using the radio-carbon (c^{14}) method with 4 hour incubation periods. He determined that primary productivity was apparently greater when determined from short-term rather than from 12 to 24 hour incubation periods. Two to 4 hour incubation periods were used during this study, and the resulting depth profiles of gross primary productivity for different time periods throughout the day were very similar to those reported by Rhode. The gross production of oxygen in relation to depth and time of day in the Arcata pond on July 28, 1961 is indicated in Figure 30. As shown, depth profile of gross production can be divided into three general areas: light-optimum, light-inhibited, and light-limited. These areas are defined as follows: (1) light-optimum--that part of a single water column in which the optimum or maximum rate of gross oxygen production is occurring at any given time, (2) light-inhibited--that part (near the surface) of the water column in which the rate of gross oxygen production is less than at some greater depth, (3) light-limited--that part of a water column below the light optimum zone in which gross oxygen production is less than in the light optimum zone. In the Arcata pond, when surface light intensity was below approximately 0.5 ly/min. only a light-limited area of productivity was observed to be present. When surface light intensity exceeded 0.5 ly/min. all three areas of oxygen production, as defined above, were present. On July 28 (Figure 30A) the depth of the euphotic zone extended to 70-80 cm. and light inhibition of photosynthesis was apparent at depths up to 25 cm.

Planimetry was used on depth profiles plotted from gross oxygen production values observed at four depths in the pond (as shown in Figure 30) to obtain "mean" rates of gross oxygen production per cubic meter (mg O_2/M^3 mn The mean gross oxygen production rates for June 21 and July 28 were plotted against time in Figures 31 and 32, respectively, and the mean concentrations of Chlorophyll a/M^3 and solar radiation for the same days were also plotted in these figures. Graphically, the mean gross production of oxygen-time curve corresponds in shape to the curve representing surface light intensity on clear days (Figures 31 and 32). The maximum rate of mean gross oxygen production on a clear day usually occurred at about noon, but it can occur at a later time of day if a fog or cloud cover is present in the morning. The largest mean concentration of Chlorophyll a occurred sometime after the maximum rate of gross oxygen production. On June 21 gross oxygen production was approximately 17 grams of oxygen/M^3/day, or about 140 lbs/acre/day.

Relationship Between the Concentration of Chlorophyll a and Gross Oxygen Production

In a survey of the literature, Strickland (1960) reports for marine waters an hourly rate of primary production (when the photosynthetically

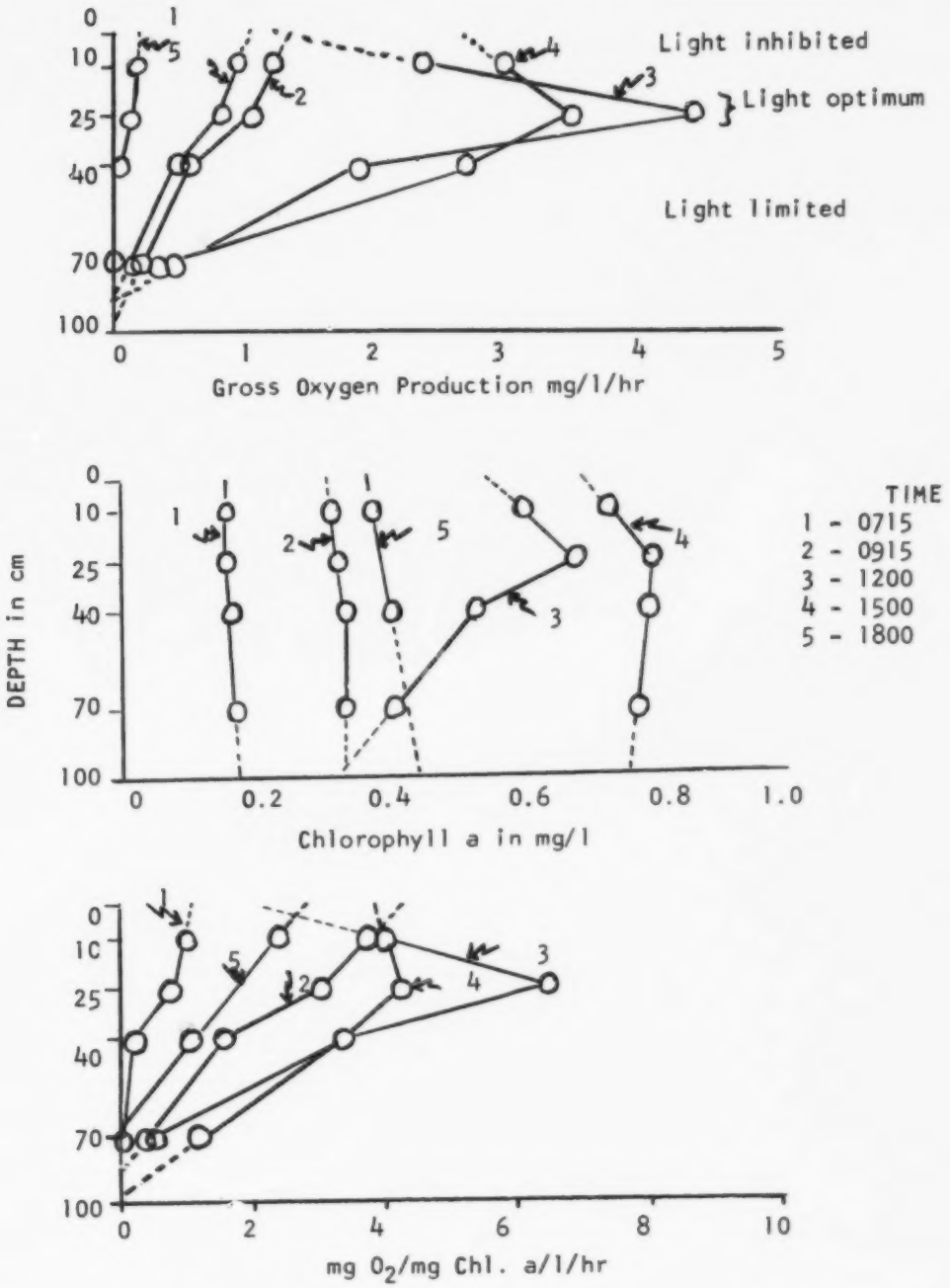

Figure 30. A, gross oxygen production in relation depth; B, Chlorophyll a in relation to depth; C, gross oxygen production per mg of Chlorophyll a at different times of day on July 28, 1961.

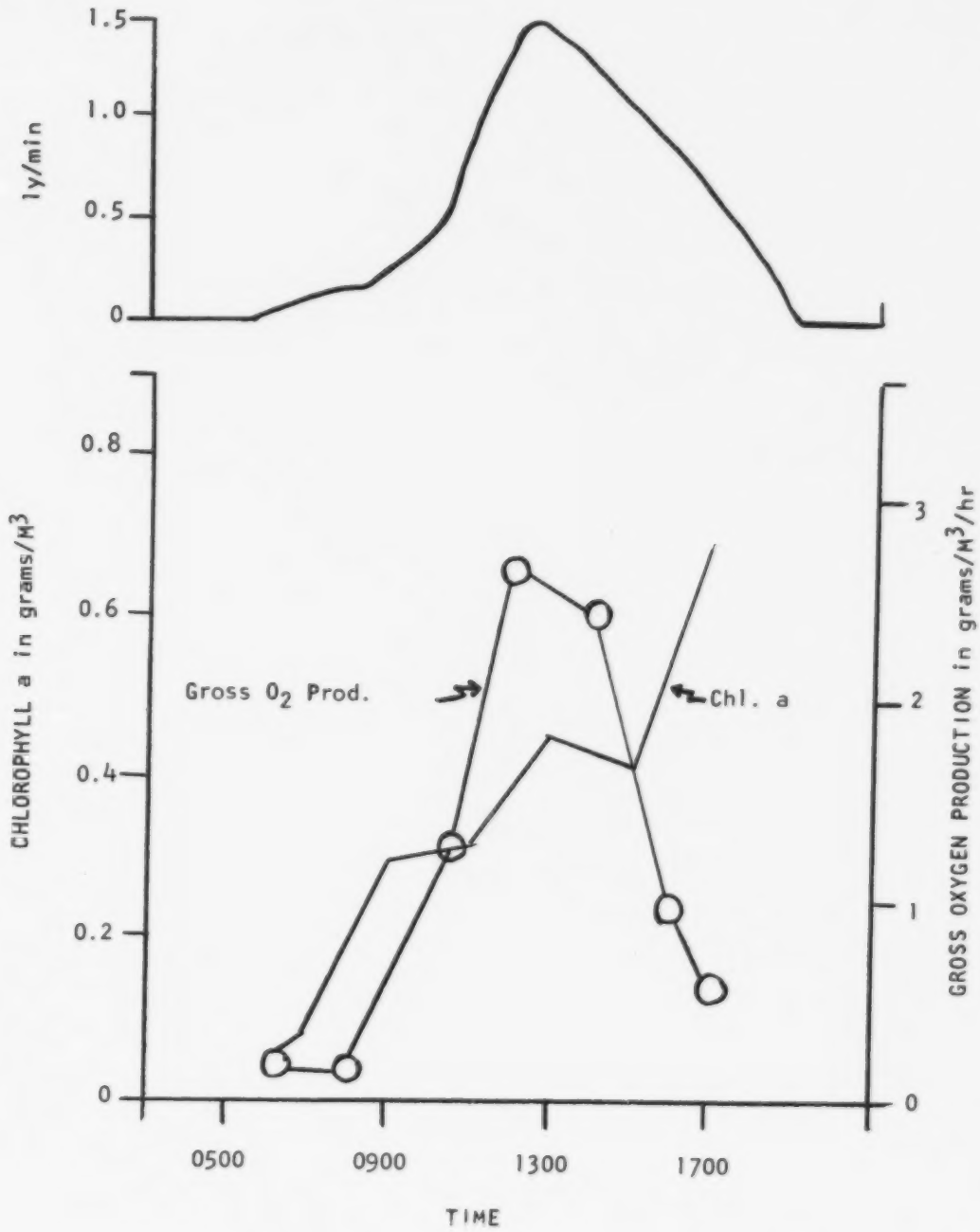

Figure 31. The gross production of oxygen in relation to the concentration of Chlorophyll a and solar radiation at different times of day on June 21, 1961

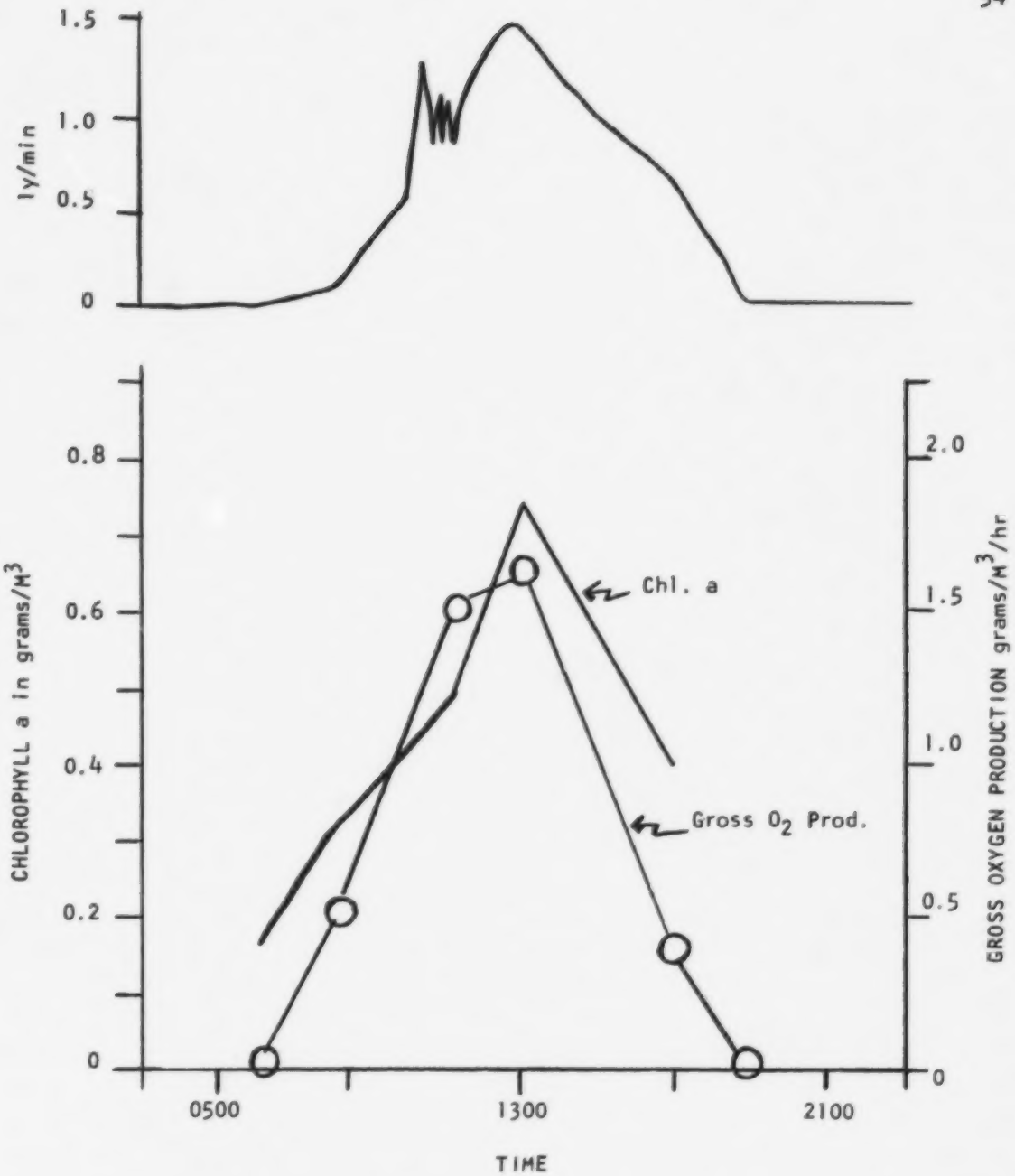

Figure 32. The gross production of oxygen in relation to the concentration of Chlorophyll a and solar radiation at different times of day on July 28, 1961.

active light intensity is about 0.1 ly/min) of about 1-10 mg carbon/mg Chl a/l/hr (3-30 mg O /mg Chl. a/l/hr) with an average rate of .4 mg carbon/mg Chl. a/l/hr (12.8 mg O_2/mg Chl. a/l/hr). In the Arcata pond, maximum gross photosynthetic rates observed were 12.5 mg O_2/mg Chl. a/l/hr on July 28 at surface light intensities of approximately 0.7 ly/min (3200-7600 angstrome). The depth profiles of oxygen production per mg of Chlorophyll a (Figure 30) and the curves of gross production of oxygen (Figure 30A) are very similar in shape. Variations in the production of oxygen per mg of Chlorophyll a in relation to surface light intensity and depth in the pond were observed on a clear day (see Figure 33). The observed rates of oxygen production per mg of Chlorophyll a were never as great at 10 cm as the rates observed at 25 cm; however, this was undoubtedly the result of discontinuous observations. Probably for some short time during mid-morning and mid-afternoon the photosynthetic rate was just as great or greater at the 10 cm depth as the maximum rate observed at 25 cm. Figure 33 illustrates the observed relationship between oxygen production per mg of Chlorophyll a and surface light intensity at the four sampled depths. With the exception of the observations at the 10 cm depth, these curves are believed to generally represent what really occurred.

Community Respiration per mg of Chlorophyll a

Community respiration in the Arcata pond was estimated from measured decreases in dissolved oxygen in incubated Dark bottles (LB-DB). The *in situ* incubation periods for the Dark bottles were from two to four hours. The concentration of Chlorophyll a was determined in aliquots of water samples used to fill the Dark bottles. The water samples from which Dark bottle and Chlorophyll a determinations were made were collected at four different depths in the pond at different hours of day between sunrise and sunset. Linear regressions, determined by the method of Simpson, Roe, and Lewontin, (1960), of community respiration against Chlorophyll a were determined for two differerent days. These regression lines and the correlation coefficients (r) for respiration rate per mg Chlorophyll a (resp. O /mg Chl. a/l/hr) on June 21 and July 28 are shown in Figure 34. The calculated line for June 21 was a poor fit to the points (rm0.57), but a high correlation coefficient (r=0.92) was observed for the data of July 28. The water temperatures were 18 to 22° on June 21, and 18.5 to 21.5° on July 28. On both days, the concentrations of Chlorophyll a were about the same.

The characteristics of the regressions for the two sampled days were quite different. Assuming that r^2 is a measure of the degree to which changes in the concentration of Chlorophyll a are determined by changes in the respiratory rate, on July 28 84 per cent of the change in the rate of community respiration could be attributed to changes in the concentration of Chlorophyll a, but on June 21 only 32 per cent of the change in a respiration rate could be explained by variation in the abundance of Chlorophyll a. Since the recorded respiration rates represent community respiration and not phytoplankton respiration alone,

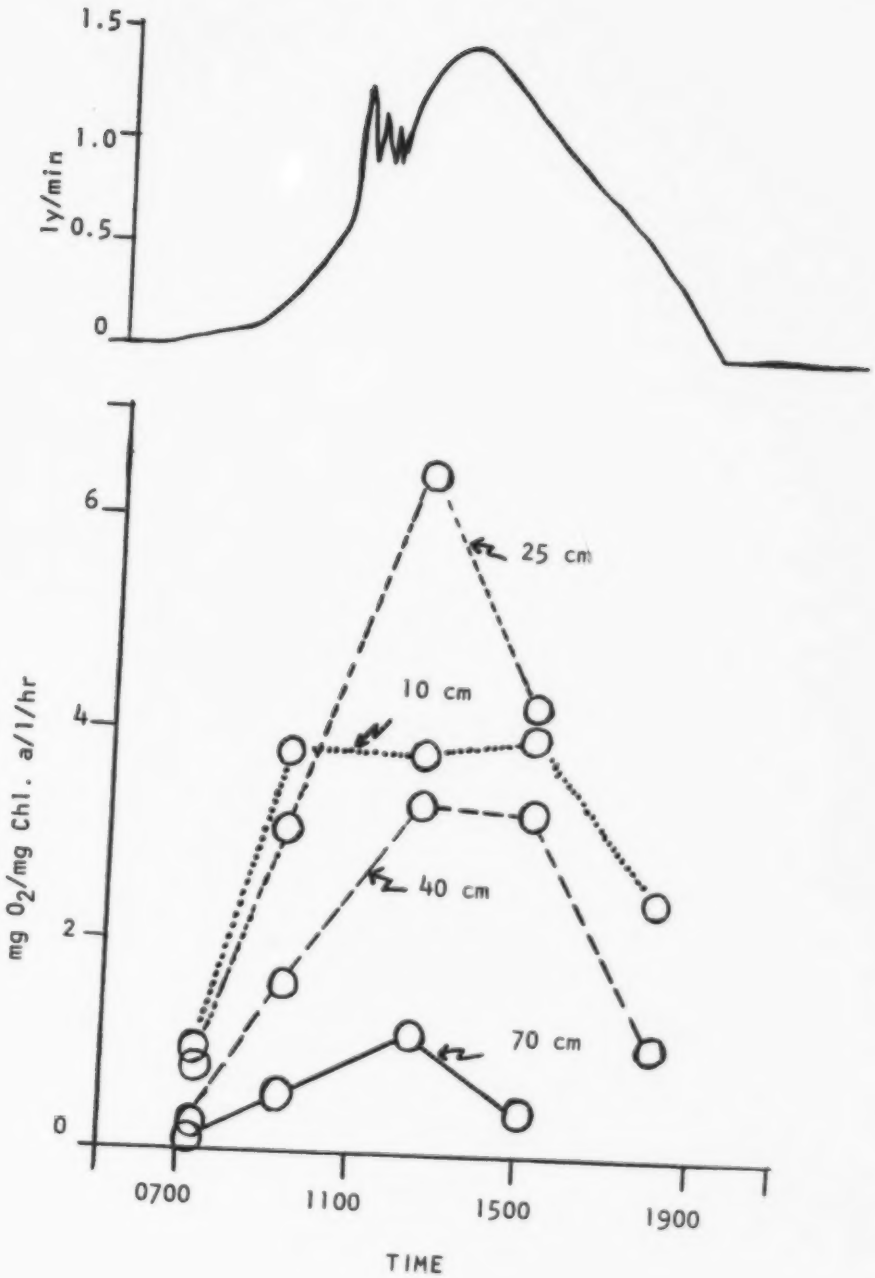

Figure 33. Gross production of oxygen per mg of Chlorophyll a at four depths in relation to solar radiation at different times of day on July 28, 1961

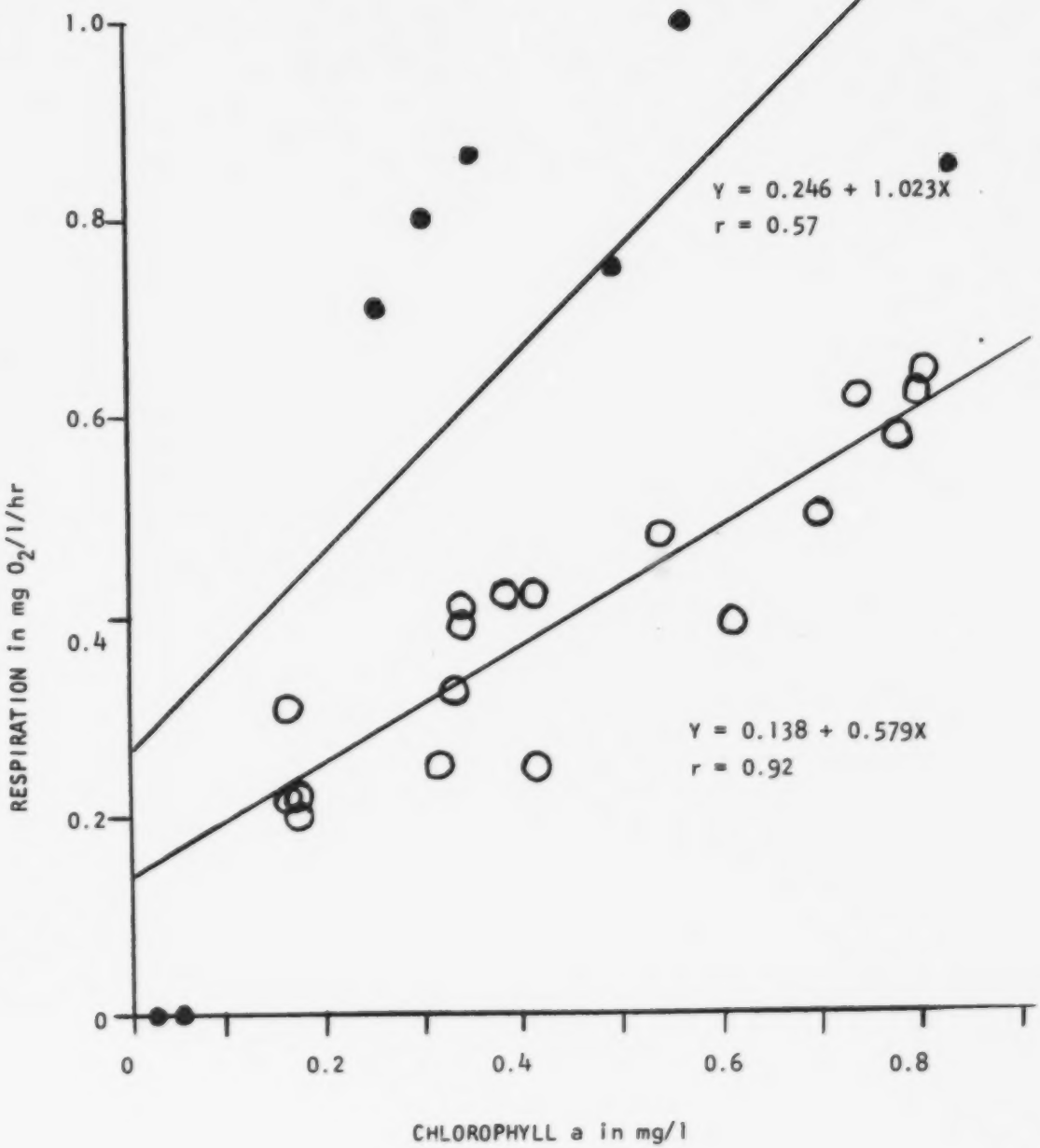

Figure 34. The relationship between community respiration and the concentration of Chlorophyll a on different days, June 21 and July 28, at similar water temperatures, 18-22 and 18.5 -21.5 degrees centigrade, respectively.

the oxygen utulized in the samples by organisms other than phytoplankton must have been markedly different between these two days, especially since water temperatures were nearly the same. Cladocerans and ostracods were excluded from the dark bottles by filtering the samples through a 6 mesh cloth; however, no attempt was made to estimate how many bacteria, protozoan, rotifers, etc, may have remained in the samples, and from microscopic examinations of water samples it was determined that the abundance of these organisms varied widely from one day to another.

Using the calculated linear regression formulae, the mean hourly rate of oxygen consumption per mg of Chlorophyll a on each of the two days of observation was estimated. On June 21 community respiration was 1.24 O_2/mg Chl. a/l/hr. On July 28 it was 0.72 mg O_2/mg Chl. a/l/hr. Since the data of July 28 had a correlation coefficient (r) of 0.92, the observed respiratory rate of 0.72 mg O_2/mg Chl. a/l/hr. may have been a fair estimate of the rate of O_2 consumption by phytoplankton.

DISCUSSION

Since dissolved oxygen is required for the aerobic decomposition of domestic sewage and since such decomposition is one of the principal processes resulting in the treatment of sewage in oxidation ponds, adequate dissolved oxygen must be available at all times in such ponds if aerobic decomposition is to be maintained at high rates. When sufficient dissolved oxygen is not present most decomposition is anaerobic, and the odorous gasses produced by such decomposition may become a public nuisance. Two of the major causes of extensive anerobic conditions in seage oxidation ponds are: inflow of organic matter that exceeds the treatment capacity of a pond, and reduction of the phytoplankton in a pond to such a low level of abundance that it is unable to meet, through photosynthesis, some of the oxygen demand of the aerobic sewage decomposition processes.

Since other sources of oxygen are ordinarily insufficient to meet the need for oxygen in an oxidation pond, photosynthetically produced oxygen is usually necessary; for proper operation of a pond. Most photosynthetic oxygen in oxidation ponds is produced by phytoplankton. Thus, the size, composition, and other features of phytoplankton populations, and the factors that influence the production of oxygen by them are important in the maintenance of suitable oxygen conditions in these ponds. The main objective of this study was to gain knowledge of the dynamics of phytoplankton populations and of some of the factors that influence changes in phytoplankton in sewage oxidation ponds so that observed changes in dissolved oxygen conditions might be understood and explained. The characteristics of the phytoplankton populations and many of the environmental factors which regulate the production of oxygen by these populations in sewage oxidation ponds are probably similar to those in many other freshwater ponds in regions with similar climatic conditions. Therefore, many of the results of studies of oxidation ponds probably may be applied to the understanding of the ecology of shallow, very rich ponds in general.

The concentration of Chlorophyll a was chosen as the index to the abundance of phytoplankton in the Arcata pond. Changes in the concentration of Chlorophyll a may indicate changes in the abundance of phytoplankters, but they may not very well indicate the rate of growth of a population. The net rate of oxygen production by a population of phytoplankton is believed to be proportional to the rate of growth of the population. In the Light and Dark Bottle method of extimating oxygen production, the respiration observed in the Dark Bottle in community respiration rather than respiration by phytoplankton alone; consequently, an accurate estimate of net oxygen production sometimes may be unattainable with this method. Because of such possible inaccuracies in estimating phytoplankton respiration and the resulting errors in calculating net oxygen production, gross oxygen production was considered in this study to be as reliable an estimate of the rate of plant growth as was net production. Gross oxygen production measurements were made

in conjunction with determinations of the concentration of Chlorophyll a. In this way some indication of the rates of plant growth at different light intensities, water depths, and levels of phytoplankton abundance could be obtained. Although surface light intensity measurements were made, the equipment needed to measure sub-surface light intensity was not available. An estimate of the quantity of photosynthetically active light at a specific water depth can be made from the results of a Light and Dark Bottle experiment. Strickland (1960) reports the optimum light intensity for photosynthetic activity to be 0.1-0.15 ly/min. Using the observed oxygen production per mg of Chlorophyll a obtained from this study, it was found that in certain cases the depth of maximum oxygen production was also the depth of optimum gross oxygen production per unit of Chlorophyll a, and also the depth of optimum light intensity. Under the conditions of the present study, when photosynthesis is inhibited by light intensity near the surface and the zone of maximum oxygen production occurs at some depth below the surface, it may be assumed that at this depth the light intensity is approximately 0.1-0.15 ly/min.

After some preliminary estimates of the gross production of oxygen and the concentration of Chlorophyll a had been made, it was decided that a long-range sampling program for the study would have to be designed to measure very short-term changes in the abundance of phytoplankton. Preliminary sampling and determinations of Chlorophyll a indicated that this pigment was not uniformly distributed vertically or horizontally, and that the total amount of this pigment in the pond could change radically within a period of a few hours. It was decided, therefore, that the general pattern of change in the concentration of chlorophyll during a single day would have to be ascertained before a sampling program to estimate differences in the abundance of phytoplankton on a day to day basis could be established.

From the results of this study, it has been determined that when a description of the dynamics of a population of phytoplankton is the primary objective, the frequency of sampling, the time of day when the samples are collected, and the depths of collection are of prime importance. When long-term observations on phytoplankton dynamics are to be made, a minimum of one sample each day should be collected. Less frequent sampling may provide misleading data on the changes that have taken place in the abundance of phytoplankters when a smooth curve is to be drawn between points representing the observations. The time of day of sample collection is extremely important, because the rate of change in the size of the phytoplankton population is exponential when conditions are favorable. Thus, within a short period of time at favorable times of the day the size of the population may change by several magnitudes. It is desirable to collect each sample as near as possible under a set of standard conditions. During daylight hours, several variables influence the rate of change of population size, these are: changing light conditions, mixing rates, the general level of abundance of phytoplankton, the general level of plant nutrients, and the general physiological conditions of the phytoplankters. Because of these and other variables, the best sampling time is probably shortly

before sunrise. The population of phytoplankton at this pre-daylight hour represents the "seed" for what occurs during the following daylight hours. The water surface, which is the zone of maximum production during early morning, is probably the best depth for sample collections. Samples collected at the surface before sunrise probably represent an average minimal population from day to day. This schedule and depth of sampling reduces variations in the concentration of Chlorophyll a that are primarily the result of an environment regulated by light intensity. The concentration of Chlorophyll a in a single sample taken at any one particular time or depth would probably not be a good estimate of the sewage concentration in an entire pond over that entire day. In summary, an adequate sampling program, designed to measure the changes in abundance of phytoplankton in a sewage oxidation pond or a similar body of water, must include considerations of time of day and water depth at which samples are to be taken.

On clear days the concentration of Chlorophyll a in the oxidation pond usually increased rapidly during the morning hours. After reaching a peak in abundance between 1300 and 1500 hours, the concentration decreased during the remainder of the day. However, this pattern changed with the occurrence of fog or a cloud cover. When there was a cover of fog during the morning hours, an accellerated rate of increase in the concentration of Chlorophyll a did not occur until the fog was gone, usually by 0900-1000 hours. In effect this reduced the length of time that the phytoplankton population was influenced by high levels of light intensity. In such cases, Chlorophyll a did not reach a peak in concentration at midafternoon, but was still increasing in concentration late in the day. When a low level of light intensity prevailed throughout the day, there was a general decrease from the early morning level in the concentration of Chlorophyll a through-out the day; however, if the light intensity became relatively high for a short period of time during a cloudy day, there was a corresponding increase in the concentration of Chlorophyll a.

The principal biological factors that regulate the vertical distribution of phytoplankton (Chlorophyll a) are reproduction, growth, mortality, and motility. The principal physical factors regulating much distributions are light intensity, settling, and mixing. From the results of this study, light intensity appeared to be the most important of the physical factors. At low surface light intensities, such as occur during the early morning and late afternoon, the distribution of Chlorophyll a in the pond was almost the same from the surface to the bottom. At higher light intensities a greater proportion (up to 80 per cent) of the Chlorophyll a was found in the upper 50 cm of the water column. With surface light at mid-day intensities, a greater proportion of the Chlorophyll a in a column of water was distributed between the depths of 10 and 40 cm.

The observed maximum ratio of oxygen production during early morning and late afternoon in the water column were at the surface. During mid-day, the maximum observed rates of oxygen production were often at

some depth below the surface. The rate of production was always lower at depths over 40 cm than at any lesser depth at any given time. The depth at which oxygen production was just sufficient to meet the oxygen demand of community respiration was generally about 70 cm. The highest observed mean rates of gross oxygen production in an entire column of water occurred at mid-day. Based on these observations, vertical zones of oxygen production as influenced by light intensity can be established. These zones are herein designated as: light-inhibited, light-limited, and light-optimum. One, two, or all three of these zones may exist at any one time; however, when there is a light-inhibited zone all three are necessarily present.

Motility was an important factor in determining the vertical distribution of Chlorophyll a when the most abundant phytoplanktor present was the motile flagellate, Euglena. In a survey of the literature, Bendix (1960) found that Euglena tends to migrate (presumably) toward the zone of optimum light intensity for photosynthetic activity within their environment. Such response to light intensity may have been very significant in determining the vertical distribution of Chlorophyll a in the Arcata pond. Since live cells settling from the water mass were not separable from the settling dead cells in the collected samples, mortality and settling were indistinguishable in their total effect on altering the size of the population of phytoplankton as indicated by measurements of the concentration of Chlorophyll a. Precise rates of vertical mixing in the Arcata pond are unknown; however, using dye, it has been determined (unpublished data) that vertical mixing in the entire pond is usually virtually complete in less than six hours.

The ways in which the principal physical and biological factors may hypothetically determine the vertical distribution of chlorophyll a, at two levels of surface light intensity, are graphically represented in Figure 35. This hypothetical analysis was based in part on observations made during this study and partly from information derived from the literature.

Part A of Figure 35 illustrates a uniform distribution of chlorophyll from the surface to the bottom of a hypothetical pond when the surface light intensity is about 0.5 ly/min. Under these conditions, the depth profile of the gross production of oxygen is usually found to be of an exponential nature as the figure shows. The effects of reproduction and growth in increasing the observed concentration of chlorophyll, and therefore on the vertical distribution of chlorophyll, are thought to decrease with depth in a manner proportional to the decrease in the rate of gross oxygen production. Certain motile algae tend to move toward the region of the water column where optimum light conditions for photosynthetic activity exist. In this hypothetical case, this movement would be toward the surface. This phototactic response to light intensity may be more pronounced in some organisms than in others depending on physiological differences between organisms. Some live phytoplankters which settle to the bottom during the night may be stimulated by light to migrate from the bottom toward the surface. A

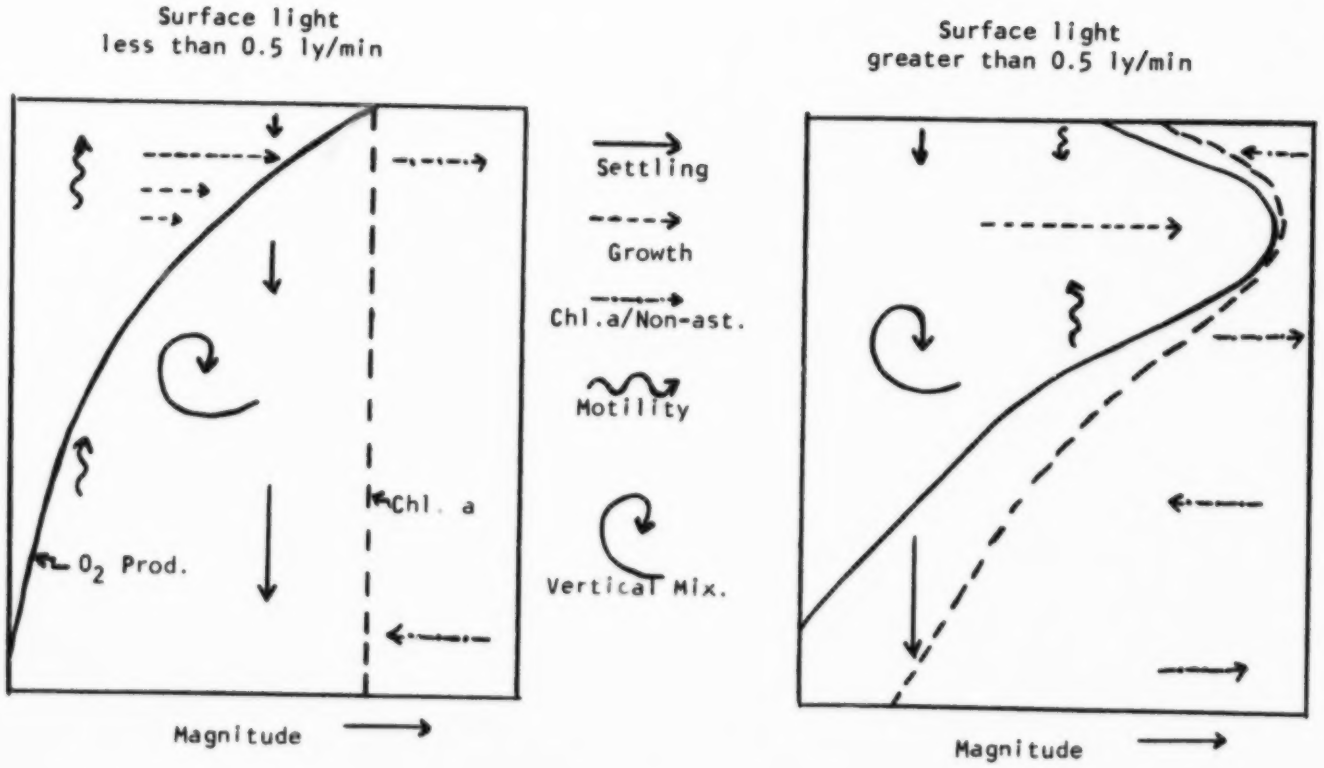

Figure 35. A hypothetical illustration of some of the factors which determine the vertical distribution of chlorophyll. The symbols represent the general area, direction, and magnitude of these influences. The length of the symbol-lines represents the relative rate of change.

migration of this type would tend to increase the overall concentration of chlorophyll in the water column. Due to motility and the formation of new individuals through growth and reproduction, a predominately younger population of phytoplankters may accumulate in the upper parts of the water column, and a higher proportion of scenescent organisms, which have settled from the surface region, may be expected to be present nearer the bottom. Vertical mixing aids in the development and maintenance of a uniform distribution of chlorophyll.

Part B of Figure 35 illustrates the vertical distribution of chlorophyll when surface light intensities are greater than 0.5 ly/min. The shapes of the curves representing the depth profiles of both chlorophyll and the gross production of oxygen are almost the same. Both show unequal distribution with depth. The depth at which the highest rate of oxygen production is occurring is also the depth of the largest concentration of chlorophyll. It is assumed that the pattern of motile movement at the higher light intensities is different from that occurring when surface light intensity is less than 0.5 ly/min. Since movement among motile phytoplankters tends to be toward the region of optimum light intensity, those organisms near the surface would move deeper in the water column as the depth of optimum light intensity moved downward. This response to supra-optimal light intensity would tend to decrease the concentration of chlorophyll near the surface and increase it at the depth of optimum light. Another factor which would tend to accentuate the level of abundance of chlorophyll at the depth of optimum light intensity is the rate of growth and reproduction at such a depth. Gross oxygen production per unit of chlorophyll is less both above and below this optimum depth. Over a period of time, a difference in the rate of growth above and below the depth of optimum light intensity would create a non-uniform distribution of phytoplankton with respect to depth. In addition to the above influences on the vertical distribution of chlorophyll, there may be a decrease in this pigment near the surface due to photooxidation of chlorophyll (Rabinovitch, 1945). In the hypothetical case being discussed, all of these factors influence the vertical distribution of chlorophyll and tend to concentrate this pigment at a depth of optimum photosynthetic activity. The effectiveness of vertical mixing in creating a uniform vertical distribution is probably usually overshadowed by the other factors previously mentioned that invluence vertical distribution.

One of the major factors influencing the abundance of phytoplankton in the Arcata pond over a long period of time, such as 30 days, was the abundance of Daphnia. When present, rotifers may have much the same effect on the phytoplankton population as do Daphnia. Although numerous at times, rotifers did not occur in large numbers during the time of collection for Daphnia. Estimates of population size were made only for Daphnia. The numbers of Daphnia in the pond varied from nearly zero to about 400 per liter. A correspondence was found between the abundance of Daphnia and Chlorophyll a. When there was a peak in the population of Daphnia there was a minimum concentration of chlorophyll, and vice versa. At extremely high levels of abundance of Daphnia, the

concentration of Chlorophyll a approached zero, and the water usually became clear enough for the bottom of the pond to be clearly seen. At such times, the production of photosynthetic oxygen was minimal, and in addition, there was a great oxygen demand due to the respiration of Daphnia; consequently the pond became anacrobie or nearly so. After the oxygen had been depleted, the Daphnia would undergo mass mortality, apparently due to asphyxiation. Shortly thereafter, increases in phytoplankton would occur resulting in a return to aerobic conditions in the pond. This pattern of events often repeated itself several times in a summer. Reduction of the population of phytoplankton, by "grazing" by filter feeding zooplankters, is probably the most important single cause of anaerobic conditions in the Arcata pond.

In any attempt to measure long-term (greater than one day) changes in the abundance of phytoplankton in a shallow and enriched pond, it is necessary to consider short-term (one day or less) changes in abundance. It has been pointed out in this thesis that short-term changes may be nearly as large as changes that will occur over longer periods of time. Corresponding diurnal variations in the extracellular environment, such as those of dissolved oxygen and pH, are well recognized, thus the same type of planning must be used in the study of the dynamics of phytoplankton as that used in establishing the oxygen characteristics of a stream or lake. In some cases, the filter-feeding zooplankton may control long-term variations in the abundance of phytoplankton; consequently, when assessing such long-term variations, it is necessary to consider relationships between the levels of abundance of zooplankton and phytoplankton.

SUMMARY

1. The fluctuations of the populations of phytoplankton in a 50 surface acre sewage oxidation pond were determined during 1960 and 1961 by measuring concentrations of Chlorophyll a at selected stations and depths.

2. Concentrations of Chlorophyll a were measured at four depths at each of several stations in the pond. From observed differences in the concentrations at these depths, it was determined that the vertical distribution of Chlorophyll a was quite variable over even a short period of time, and that the vertical distribution existing at any specific time was determined largely by surface light intensity. At surface light intensities of about 0.5 ly/min and less, Chlorophyll a was distributed nearly evenly from the surface to the bottom. At mid-day light intensities greater than about 0.5 17/min. the greatest concentration of Chlorophyll a occurred between the depths of 25 and 40 cm. Fifty to 80 per cent of the total concentration of Chlorophyll a, at mid-day on a clear day in July in a water column of one cubic meter, occurred in the upper 50 cm of the column.

3. The concentration of Chlorophyll a per cubic meter of pond water was found to vary greatly during the daylight hours. Concentrations varied from 1 gram/M^3 (early in the morning) to 7.8 grama/M^3 (in mid-afternoon). Daily variations in the concentrations of Chlorophyll a per cubic meter followed patterns that were related to changes in surface light intensity.

4. At surface light intensities greater than 0.5 ly/min. there were three characteristic zones of oxygen production. These were the light-inhibited, light-optimum, and light-limited zones. These zones are defined as follows: (1) light-optimum--that part of a single water column in which the optimum or maximum rate of gross oxygen production is occuring at any given time, (2) light-inhibited--that part near the surface of the water column in which the rate of gross oxygen production is less than at some greater depth, (3) light-limited--that part of a water column below the light-optimum zone in which gross oxygen production is less than in the light-optimum zone. At mid-day on clear summer days, the light-optimum zone occurred at depths between 25 and 40 cm. The entire euphotic zone was usually less than 70 cm in depth.

5. Chlorophyll a: Non-astacin (carotenoid) pigment ratios (Chl. a/Non-ast.) at different levels of abundance of phytoplankton were determined. Changes in the ratio were found to vary inversely with changes in the mean concentration of Chlorophyll a in an entire water column. The ratio at depths of 10 and 25 cm varied directly with changes in the concentration of chlorophyll at those depths; however, variations in this ratio did not follow any specific pattern at depths of 40 and 70 cm. At mid-day, the segment of the phytoplankton population at the zone of optimum light intensity appeared to have a higher

Chl. a/Non-ast. ratio than those segments of the population in zones where photosyntehesis was restricted by light intensities that were either too high or too low.

6. Daphnia magna play a significant role in regulating the abundance of phytoplankton in the sewage oxidation pond. During periods when Daphnia was abundant (often in excess of 300 per liter), the concentration of chlorophyll, because of grazing by Daphnia, was often nearly zero. On the other hand the abundance of Daphnia appeared to be regulated to a considerable extent by the abundance of phytoplanktonic food organisms. Peaks in abundance of Daphnia often occurred shortly after peaks in abundance of phytoplankton. The virtual elimination of phytoplankters by grazing by large numbers of Daphnia was followed quickly by a drastic reduction, due to asphyxiation and starvation, in the abundance of Daphnia. Consequently, there was an oscillatory relationship between the abundance of Daphnia and the concentration of chlorophyll during the study period.

7. Chlorophyll a: ash-free dry weight of suspended solids ratios ranged between 0.0013 and 0.0138. The ratios obtained compared favorably with similar ratios which have been reported for other studies. The correlation coefficients (r) of Chlorophyll a va. ash-free dry weight, for samples taken near the outfall and in inflow, were G.87 and 0.64 respectively. Phytoplankters composed a greater proportion of the organic matter in the water at the cutfall than near the inflow.

8. The maximum observed rate of gross oxygen production per milligram of Chlorophyll a was 12.6 mg O_2/mg Chl. a/l/hr. This rate is close to previously reported maximum rates. Oxygen production per unit mass of chlorophyll a in the surface waters was less than the maximum rate occurring at more greater depth in the water column when the surface light intensity exceeded about 0.5 ly/min.

9. It was indicated by comparing the results of the Light and Dark Bottle analyses and the Chlorophyll a experiments that short-term changes in the concentration of Chlorophyli a are primarily the direct result of photosynthetic activity.

10. Total community respiration changed in direct proportion to the concentration of Chlorophyll a. The mean respiration rates during two different day times were 1.24 mg O_2/mg Chl. a/l/hr. and 0.72 mg O_2mg Chl. a/l/hr.

11. Because of wide variations in the average concentration and the vertical distribution of Chlorophyll a that can occur during any one day, conclusions on changes in the abundance of phytoplankton may be faulty if the chlorophyll sampling program does not take into account the depth and time of sample collections. To minimize the effects of light-induced short-term variations, it is suggested that samples of phytoplankton for chlorophyll analysis be taken from the surface just before day-

break when the purpose of sampling is the estimation of long-term changes in the abundance of phytoplankton.

12. A hypothetical situation, involving the factors of phytoplanktonic growth, reproduction, motility, morcality, settling, mixing, and responses to light, is presented to illustrate the roles played by these factors in the establishment of two observed types of vertical distribution of Chlorophyll a.

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Appendix A. The complete tabulation of samples for the pigments Chlorophyll a, b, c, and the Non-astacin pigments. Depth is given in either cm or S (surface) or B (bottom).

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M^3	Chl. b Mg/M^3	Chl. c MSPU/M^3	Non-ast. MSPU/M^3	Chl.a Non-ast.
2-25-60	-	H	S	521	97	-	-	-
2-25-60	-	I	S	282	53	-	-	-
2-27-60	-	H	S	366	14	8	-	-
3- 3-60	-	H	S	19	5	-	-	-
3- 3-60	-	I	S	69	28	-	-	-
3- 8-60	-	H	S	7	1	1	-	-
3- 8-60	-	I	S	12	2	-	-	-
3-12-60	-	H	S	7	+	-	-	-
3-12-60	-	I	S	6	-	-	-	-
3-17-60	-	H	S	44	5	10	-	-
3-17-60	-	I	S	54	6	40	-	-
3-24-60	-	H	S	368	48	84	-	-
3-24-60	-	H	S	368	33	7	-	-
4- 2-60	-	H	S	414	2	-	-	-
4--2-60	-	I	S	368	4	-	-	-
4- 7-60	-	H	S	82	-	-	-	-
4- 7-60	-	I	S	58	+	-	-	-
4-14-60	-	H	S	318	-	-	-	-
4-19-60	-	H	S	369	-	-	-	-
4-21-60	-	H	S	360	-	-	-	-
4-21-60	-	D	S	205	-	-	-	-
4-30-60	-	H	S	2	1	10	-	-
4-30-60	-	D	S	7	4	25	-	-
5- 5-60	-	H	S	109	16	31	-	-
5- 5-60	-	D	S	142	20	47	-	-
5-12-60	-	A	S	636	90	113	-	-

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M3	Chl. b Mg/M3	Chl. c MSPU/M3	Non-ast. MSPU/M3	Chl. a Non-ast.
5-12-60	-	D	S	466	55	391	-	-
5-12-60	-	B	S	607	78	57	-	-
5-12-60	-	H	S	338	39	64	-	-
5-19-60	-	H	S	762	38	15	-	-
5-28-60	-	H	S	6	2	3	-	-
6- 2-60	-	H	S	9	4	19	-	-
6- 2-60	-	H	B	10	2	4	-	-
6- 2-60	-	D	S	21	9	30	-	-
6- 2-60	-	E	S	7	2	18	-	-
6- 7-60	-	H	S	12	2	5	-	-
6- 7-60	-	H	B	16	4	10	-	-
6- 7-60	-	D	S	11	4	5	-	-
6- 7-60	-	D	B	16	5	16	-	-
6- 9-60	-	H	S	5	2	8	-	-
6- 9-60	-	D	S	25	6	15	-	-
6- 9-60	-	C	S	8	2	6	-	-
6- 9-60	-	E	S	13	3	8	-	-
6-20-60	-	A	S	384	155	-	-	-
6-20-60	-	E	S	391	256	2	-	-
6-20-60	-	D	S	244	278	-	-	-
6-23-60	-	H	S	340	51	43	-	-
6-23-60	-	E	S	494	64	-	-	-
6-23-60	-	D	S	284	244	-	-	-
6-27-63	-	H	S	608	93	-	-	-
6-27-60	-	D	S	1012	182	-	-	-
6-29-60	-	H	S	678	110	-	-	-
6-29-60	-	D	S	699	112	-	-	-
7-11-60	1320	H	10	600	130	186	-	-
7-11-60	1415	D	10	481	100	-	-	-

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M3	Chl. b Mg/M3	Chl. c MSPU/M3	Non-ast. MSPU/M	Chl. a Non-ast.
7-14-60	1035	H	10	461	135	49	-	-
7-14-60	935	D	10	393	132	62	-	-
7-19-60	1110	H	10	425	122	74	-	-
7-19-60	1100	D	10	349	73	36	-	-
8- 1-60	1335	H	10	278	65	-	-	-
8- 1-60	1335	E	10	319	56	-	-	-
8- 1-60	1405	J	10	222	34	-	-	-
8- 1-60	1430	D	10	103	50	-	--	-
8-12-60	1030	E	10	169	42	-	-	-
8-12-60	1030	E	25	177	34	-	-	-
8-12-60	1030	E	40	162	26	-	-	-
8-12-60	1030	E	70	168	17	-	-	-
8-18-60	-	H	10	545	111	-	-	-
8-18-60	-	E	10	714	113	-	-	-
8-18-60	-	J	10	586	103	-	-	-
8-18-60	-	D	10	551	76	-	-	-
8-19-60	-	E	10	96	26	13	-	-
8-19-60	-	E	25	112	26	12	-	-
8-19-60	-	E	70	197	35	-	-	-
8-30-60	-	H	10	130	46	48	-	-
8-30-60	-	E	10	126	27	4	-	-
8-30-60	-	J	10	113	28	3	-	-
8-30-60	-	D	10	104	22	5	-	-
9-15-60	-	A	10	47	12	14	-	-
9-15-60	-	A	40	39	10	13	-	-
9-15-60	-	A	100	44	11	8	-	-
9-15-60	-	A	170	37	12	7	-	-
9-15-60	-	E	10	82	27	28	-	-
9-15-60	-	E	25	94	24	19	-	-

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M3	Chl. b Mg/M3	Chl. c MSPU/M3	Non-ast. MSPU/M	Chl. a Non-ast.
9-15-60	-	E	40	98	23	25	-	-
9-15-60	-	E	70	110	28	118	-	-
9-24-60	0910	A	10	12	6	-	-	-
9-24-60	0910	A	40	16	3	4	-	-
9-24-60	0910	A	100	21	6	-	-	-
9-24-60	0910	A	200	9	1	-	-	-
9-24-60	0940	D	10	38	12	-	-	-
9-24-60	0940	D	25	31	11	-	-	-
9-24-60	0940	D	40	34	10	-	-	-
9-24-60	0940	D	70	33	9	-	-	-
9-24-60	1015	E	10	47	10	-	-	-
9-24-60	1015	E	25	46	10	-	-	-
9-24-60	1015	E	40	44	8	-	-	-
9-24-60	1015	E	70	44	13	-	-	-
10- 8-60	1000	D	10	463	48	-	-	-
10- 8-60	1000	D	25	467	44	-	-	-
10- 8-60	1000	D	40	467	49	-	-	-
10- 8-60	1000	D	70	440	42	-	-	-
10- 8-60	1030	E	10	1269	217	-	-	-
10- 8-60	1030	E	25	1324	132	-	-	-
10- 8-60	1030	E	40	1326	152	-	-	-
10- 8-60	1030	E	70	1328	113	-	-	-
10- 8-60	1040	O	-	797	105	-	-	-
10-22-60	1045	E	10	1885	277	-	-	-
10-22-60	1045	E	25	2174	382	-	-	-
10-22-60	1045	E	40	1650	227	-	-	-
10-22-60	1045	E	70	1542	188	-	-	-
11- 5-60	1030	E	10	1941	378	-	-	-
11- 5-60	1030	E	25	2344	229	-	-	-

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M3	Chl. b Mg/M3	Chl. c MSPU/M3	Non-ast. MSPU/M3	Chl. a Non-ast.
11- 5-60	1030	E	40	1075	124	-	-	-
11- 5-60	1030	E	70	1746	135	-	-	-
3-31-61	1040	A	10	224	22	-	-	-
3-31-61	1035	D	10	179	23	-	-	-
3-31-61	1025	F	10	196	21	-	-	-
3-31-61	1015	J	10	319	30	-	-	-
3-31-61	1140	E	10	267	24	-	-	-
3-31-61	0920	E	10	224	19	-	-	-
3-31-61	1100	C	10	541	39	-	-	-
4- 8-61	1030	E	10	151	18	-	-	-
4- 8-61	1230	E	10	237	18	-	-	-
4-18-60	1150	E	10	236	27	-	-	-
4-22-61	0630	E	25	33	-	-	-	-
4-22-61	0830	E	25	-	-	-	-	-
4-23-61	0930	E	25	4	-	3	-	-
5-27-60	1000	H	10	600	78	-	-	-
6- 9-61	1100	H	10	2155	372	-	-	-
6- 9-61	1120	E	10	1052	150	-	-	-
6- 9-61	1140	J	10	1357	218	-	-	-
6- 9-61	1200	D	10	1188	171	76	-	-
6-14-61	1300	0	-	b437	80	12	-	-
6-14-61	1400	D	10	292	33	88	-	-
6-15-60	1300	0	-	101	4	-	-	-
6-20-61	1330	0	-	642	136	250	-	-
6-20-61	1330	0	-	445	63	-	-	-
6-21-61	0530	E	10	54	6	86	0.07	743.00
6-21-61	0530	E	40	29	1	26	0.08	367.50
6-21-61	0700	E	10	70	6	33	1.44	48.30
6-21-61	0700	E	40	71	10	7	7.29	9.70

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M3	Chl. b Mg/M3	Chl. c MSPU/M3	Non-ast. MSPU/M3	Chl. a Non-ast.
6-21-61	0910	E	10	346	63	62	54.7	6.10
6-21-61	0910	E	40	300	45	-	80.6	3.73
6-21-61	1100	E	10	330	52	-	90.6	3.63
6-21-61	1100	E	40	492	64	-	150.0	3.28
6-21-61	1310	E	10	562	81	-	188.9	2.98
6-21-61	1310	E	40	838	127	-	293.9	2.85
6-21-61	1500	E	10	252	29	38	75.5	3.33
6-21-61	1500	E	40	859	134	-	269.8	2.18
6-21-61	1700	E	10	698	102	-	291.6	2.69
6-21-61	1700	E	40	763	108	-	296	2.62
7-18-61	1530	E	10	895	126	-	288	2.96
7-18-61	1530	E	40	610	86	-	218	2.80
7-18-61	1530	E	70	630	74	9	212	2.98
7-18-61	1930	E	10	667	84	-	198	3.37
7-18-61	1930	E	25	684	77	-	223	3.07
7-18-61	1930	E	40	698	108	-	232	3.01
7-18-61	1930	E	70	724	88	6	244	2.97
7-19-61	1500	E	10	1172	160	16	316	3.70
7-19-61	0500	E	25	1076	137	11	342	3.14
7-19-61	0500	E	40	1044	126	-	330	3.16
7-19-61	0500	E	70	1066	153	4	328	3.25
7-19-61	0730	E	10	1350	213	10	311	4.35
7-19-61	0730	E	25	1214	195	-	281	4.33
7-19-61	0730	E	40	1284	184	-	417	3.08
7-19-61	0730	E	70	1331	158	-	433	3.64
7-19-61	1000	E	10	993	155	-	273	3.64
7-19-61	1000	E	25	924	107	-	254	3.64
7-19-61	1000	E	40	935	112	-	260	3.60

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M^3	Chl. b Mg/M^3	Chl. c MSPU/M^3	Non-ast. MSPU/M^3	Chl. a Non-ast.
7-19-61	1000	E	70	798	131	-	223	3.58
7-19-61	1230	E	10	1107	203	-	248	4.46
7-19-61	1230	E	25	1283	215	64	276	4.65
7-19-61	1230	E	40	1364	235	99	296	4.61
7-19-61	1230	E	70	706	106	-	168	4.20
7-19-61	1500	E	10	943	158	54	226	4.17
7-19-61	1500	E	25	1192	196	-	281	4.24
7-19-61	1500	E	40	1051	185	497	308	3.41
7-19-61	1500	E	70	299	57	107	74	4.05
7-19-61	1730	E	10	473	56	-	102	4.63
7-19-61	1730	E	25	408	88	6	130	3.91
7-19-61	1730	E	40	463	79	-	100	4.63
7-19-61	1730	E	70	430	74	-	89	4.83
7-19-61	1930	E	10	419	60	-	102	4.12
7-19-61	1930	E	25	413	61	-	102	4.05
7-19-61	1930	E	40	422	53	-	105	4.02
7-19-61	1930	E	70	436	63	9	104	4.20
7-22-61	0300	0	-	860	149	-	205	4.20
7-22-61	0700	0	-	1450	266	215	454	3.19
7-22-61	0900	0	-	924	129	-	324	2.85
7-22-61	1200	0	-	5806	874	-	2000	2.90
7-22-61	1500	0	-	7802	1088	76	2690	2.90
7-22-61	1800	0	-	3133	646	245	1118	2.80
7-22-61	2100	0	-	2331	387	68	748	3.12
7-23-61	0045	0	-	1142	189	63	366	3.12
7-23-61	0310	0	-	1246	214	51	392	2.18
7-28-61	0615	E	10	160	33	38	31.6	5.06
7-28-61	0615	E	25	160	33	38	31.6	5.06
7-28-61	0615	E	40	166	31	33	33.4	4.97

Appendix A. (Continued)

Date	Pacific Standard Time	Sta.	Depth	Chl. a Mg/M3	Chl. b Mg/M3	Chl. c MSPU/M3	Non-ast. MSPU/M3	Chl. a Non-ast.
7-28-61	0615	E	70	173	34	42	35.8	4.83
7-28-61	0815	E	10	314	74	113	61.1	5.14
7-28-61	0815	E	25	327	64	71	64.4	5.08
7-28-61	0815	E	40	338	71	50	82.2	4.11
7-28-61	0815	E	70	337	77	82	57.2	5.89
7-28-61	1015	E	10	609	132	2	135.2	4.50
7-28-61	1015	E	25	689	138	91	160.9	4.28
7-28-61	1015	E	40	538	101	238	-	-
7-28-61	1015	E	70	412	76	35	96.6	4.26
7-28-61	1300	E	10	739	174	65	153.4	4.82
7-28-61	1300	E	25	803	177	62	195.4	4.11
7-28-61	1300	E	40	798	169	79	221.0	3.61
7-28-61	1300	E	70	779	174	93	174.0	4.47
7-28-61	1700	E	10	380	79	-	87.6	4.34
7-28-61	1700	E	40	408	78	75	92.7	4.40

APPENDIX B

The Foerst Centrifuge was reported by Creitz and Richards (1955) to retain only 69 to 86 per cent of the suspended solids retained by the "millipore" filter. Of the one set of duplicate samples, where the two methods for the deparation of suspended solids were compared in this study, the Foerst Centrifuge retained 87.6 per cent of the amount of Chlorophyll a retained by the "millipore" filter. To check on any changes in the measured concentration of pigments that may have occurred between samples dryed at 55 degrees Centigrade and at 3 degrees Centigrade, twelve aliquots of a sample of oxidation pond water were run through a membrane filter and of these aliquots, six were dried at 55 degrees Centigrade, and six were dried at 3 degrees Centigrade in the dark for 18 hours. One of the filters dried at 55 degrees Centigrade was discarded. The results of the pigment analysis from both sets of filters are tabulated in Table 1. The major differences in the estimated pigment concentrations after drying at 55 degrees Centigrade and at 3 degrees Centigrade, appeared to occur in the concentrations of Chlorophyllc and the Non-astacin and the Astacin pigments. In order to obtain some quantitative estimate of these differences, a test of the hypothesis that there was no difference between the means of the samples dried at the two different temperatures was used. A tabular "t" of 2.262 at the .05 probability level, with nine degrees of freedom, was chosen as the limits of acceptability; therefore, using the method for testing the differences between means of Simpson, Roe, and Lewontin (1960), if the "t" value was greater than +2.262 or less the -2.262 the null hypothesis would be rejected. Values of "t" greater than +2.262 were computed for Chlorophyll c (t=4.31), Non-astacin (t=3.82) and the Astacin (t-17.73) pigments. The probability of obtaining "t" values of these magnitudes is less than .05. The two drying temperatures used appear to be all right for Chlorophyll a and b, but when 55° C. is used corrective adjustments in the observed concentrations of Chlorophyll c and the Astacin and Non-astacin pigments would be necessary.

These two errors, inherent to the method used before January 1961, reduced the quantitative measure of Chlorophyll a by 15-30 per cent due to losses occurring in the use of the Foerst Centrifuge; therefore, the chlorophyll values reported for samples collected before January 1961 are minimal. The collection and analysis procedure was was constant; therefore, the proportionate error may be constant. The listed values of Chlorophyll a in mg/l probably show the true fluctuations of Chlorophyll a occurring in the pond but at a reduced quantitative value.

Table 1. The relationships of pigment determinations with residue drying at different temperatures (3 Degrees Centigrade and 55 Degrees Centigrade) overnight using a difference of means test to show significant differences that result from drying at these temperatures. Chlorophyll a and b are in mg/M^3 and Chlorophyll c, Non-astacin and astacin pigments are Millispecific pigment units ($MSPU/M^3$).

Sample No.	Temp. Deg. C.	Pigment Chl.a	Chl.b	Chl.c	Non-ast.	Ast.	Total
1.	3	41.9	6.0	10.5	1.0	10.3	70.2
2.	3	45.3	7.4	5.0	1.0	11.4	70.1
3.	3	50.0	9.7	21.0	2.2	12.5	95.4
4.	3	42.5	8.7	2.3	1.3	10.2	65.0
5.	3	46.6	7.2	12.6	0.7	12.0	79.1
6.	3	47.4	11.2	19.8	2.6	10.6	91.6
Mean (X)		46.6	8.4	11.9	1.5	11.2	78.6
Std. dev. (s)		3.06	1.89	7.55	0.76	0.88	12.48
Std. error (SX)		1.55	0.77	1.26	0.13	0.15	2.08
7.	55	43.1	9.9	30.2	3.6	3.2	90.0
8.	55	48.1	8.7	35.1	4.2	3.8	99.9
9.	55	41.7	9.3	33.5	2.4	4.0	90.9
10.	55	41.3	7.2	21.9	2.9	3.3	76.6
11.	55	46.0	10.3	26.5	3.1	3.9	89.8
Mean (X)		44.0	9.1	29.4	3.2	3.6	89.4
Std. dev. (S)		2.92	1.21	5.35	0.69	0.38	8.48
Std. error (SX)		1.30	0.24	1.07	0.14	0.08	1.69
Diff. of means "t"		.87	.71	4.31	3.82	17.73	1.63